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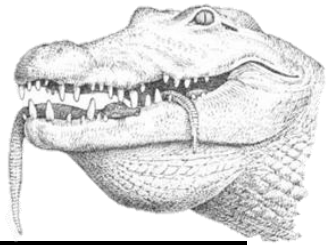
Behavioural Response to Juvenile Distress Calls as a Measure of Extended Care in Crocodilians

Lydia Dashley Giddings

Dissertation submitted to the University of Bristol in accordance with the requirements for award of the degree of Master of Science in the Faculty of Biological Sciences

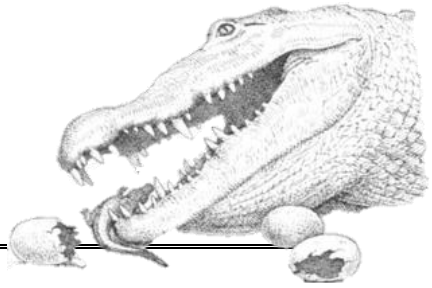
September 2019

Abstract



Crocodilians are among the last surviving members of the Archosauria clade, of which parental care is thought to be a shared trait (Coombs, 1989; Pough *et al.,* 2001). The distress calls emitted by juvenile crocodilians play an integral role in recruiting this care. At present, most research into Archosauria behaviour and acoustic communication has been conducted on birds, whilst crocodilians have been relatively neglected. It has been reported that extended care in crocodilians is modulated by the size information encoded within juvenile distress calls (Chabert *et al.,* 2015). The extent of this care and the effect that additional variables can have on behaviour has been largely ignored. Here we present evidence gleaned from playback experimentation using juvenile distress calls, that breeding period, socialness of breeding strategy, sex, species, relative body size and relatedness to the juvenile caller, can modulate crocodilian care. We propose that these variables are stronger determining factors of crocodilian response than the body size of a juvenile caller alone. This study sampled seven different species of crocodilian, four of which were categorised as Critically Endangered or Vulnerable. This is the first experimental study conducted into the extended care of male crocodilians and in the species *Tomistoma schlegelii, Crocodylus siamensis, Osteolaemus tetraspis, Paleosuchus palpebrosus* and *Crocodylus rhombifer*. We also provide the first scientific evidence of female nest guarding in *T. schlegelii*, a species that has been generally regarded as providing little or no parental care (Britton, 2009; Vitt & Caldwell, 2009). This study, and research like it, are arguably of importance as 47.8% of extant crocodilian species are either threatened or critically endangered (IUCN, 2019). More research into extended care within crocodilians could aid breeding programmes, both in the wild and captivity.

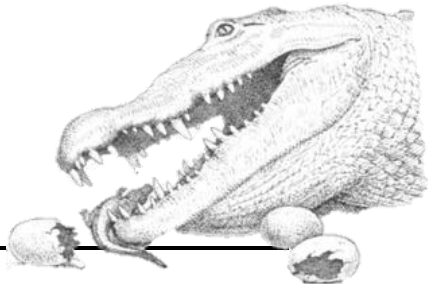
Author's Declaration



I declare that the work in this dissertation was carried out in accordance with the requirements of the University's *Regulations and Code of Practice for Research Degree Programmes* and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: ..... DATE:.....

Acknowledgements



The culmination of this research is down to the following people whom deserve recognition. Firstly, I would like to acknowledge my supervisor Dr Marc Holderied, Agata Staniewicz, Shaun Foggett and Colin Stevenson for their advice, support and kindness. Their shared love of crocodilians and the natural world was the momentum that drove this project.

I wish to express my gratitude to the Crocodile Specialist group (CSG) for their invaluable guidance and financial support. Particularly, Dr. Jeffery Lang, Nicolas Mathevon and Dr. Grahame Webb for their expert feedback.

My appreciation goes to the staff at Crocodiles of the World for accommodating much of my experimental research there, particularly Shaun Fogget and Colin Stevenson for their help.

I would like to thank the reptile keepers from Bristol Zoological Gardens Reptile Department, particularly Tim Skelton and Adam Davis, for their help whilst I was conducting my study there, both during my BSc and MRes studies.

I extend my gratitude to Michael Sweeton and Emily Grout for their expertise and encouragement throughout the process of this dissertation.

Finally, I would like to acknowledge my parents, family and friends for their unconditional love and patience.

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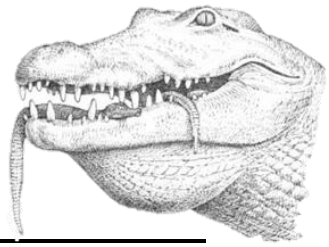

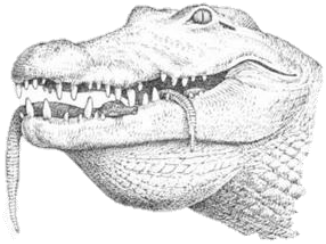

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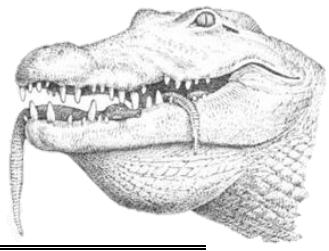
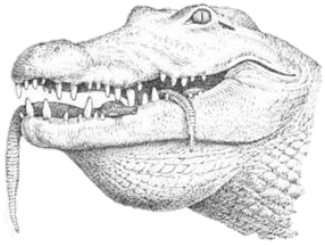


1. Introduction



Crocodilians evolved more than 250 million years ago and possess sophisticated adaptions that have enabled their continued success as top level predators (Buffetaut, 1979; Brazaitis & Watanabe, 2011). However, due to their elusive nature and nocturnal habits, a large proportion of crocodilian behaviour is unknown (Lang, 1987; Dinets, 2015a). Crocodilian numbers are declining at an alarming rate despite global efforts to increase them. An improved understanding of crocodilian behaviour will not only increase knowledge in the field of animal communication but could prevent the loss of many species. Encompassing Endangered and Vulnerable species in research could lead to effective conservation programmes, where information is specialised and targeted to the species most at risk. Additionally, an improved knowledge of crocodilian communication could lead to the development of long term, non-invasive, monitoring techniques, like those already implemented in bird conservation (Terry *et al.,* 2005; Fuller *et al.,* 2012;).

1.1 Care in Crocodilians

An understudied adaption in crocodilian behaviour is their sociality and extended parental care. Extended care is thought to be a shared trait amongst archosaurs, a group that encompasses birds, modern day crocodilians and dinosaurs (Coombs, 1989; Pough *et al.,* 2001). Currently, birds are the most extensively studied in this group. Parental care has been widely reported within the crocodilian phylum and consists of nest guarding and assisted hatching (Coombs, 1989). Following egg emergence, many parents carry offspring in their mouths to a nearby waterbody (Pough *et al.,* 2001). Females appear to be the primary care giver. However, there have been reports of biparental care in some species (Lang 1989; Tullberg *et al.,* 2002,). Seven species were sampled in this study; *Crocodylus siamensis, Crocodylus rhombifer, Crocodylus niloticus, Crocodylus porosus, Paleosuchus palpebrosus, Tomistoma schlegelii,* and *Osteolaemus tetraspis*. From the seven studied species, *C. siamensis, C. rhombifer* and *C. niloticus* have been reported to display bi-parental care (Brueggen, 2002; Conners, 2002; Tullberg *et al.,* 2002)

Extended care in crocodilians can be defined as care for young, post hatching (Tullberg *et al.,* 2002; Whitaker 2007). Behaviours have been categorised as predation protection (most commonly observed), enabling basking/transportation of young on backs and food provisioning (Brueggen, 2002; Brazaitis & Watanabe, 2011). Protective behaviour, which is characterised as chasing off or attacking predators, is

often solicited upon hearing a juvenile's distress call (Gorzula, 1978; Romero, 1983; Whitaker, 2007). There has been a higher occurrence of crocodilian care reported among pre-hatchlings than post-hatchlings (Pough *et al.,* 2001).

Predation rate is negatively correlated to body size in crocodilians (Somaweera *et al.,* 2013). Consequently, staying in close vicinity to a protective adult and within a pod of conspecifics greatly increases survival rates and population fitness (Staton, 1978; Somaweera *et al.,* 2013). Extended care in crocodilians is expected to be less energetically costly, compared to birds and mammals, as juveniles can source their own food (Pough *et al.,* 2001). It has been proposed that the risk of starvation is lower in crocodilians compared to birds and mammals, which could partially explain why food provisioning is absent (Lobaina, 2014). Incidents of intentional feeding have been observed in *Crocodylus intermedius, Caiman latirostris, O. tetraspis* and more extensively in *C. siamensis* (Brueggen, 2002; Whitaker, 2007). Observations of food provisioning mainly occurred in captivity, where resources are high and cost is low (Brueggen, 2002; Whitaker, 2007). Consequently, food provisioning may be a behaviour unique to captive living crocodilians.

The length of extended care appears to differ among species and can vary from a few weeks to over a year, at which point juveniles tend to disperse (Lang, 1987; Campos *et al.,* 2012). *C. niloticus* dispersal time can be predicted by juvenile size as adults become increasingly intolerant of intermediate sized individuals (approximately 1.2 m) (Hutton, 1989). Size related dispersal is predicted to occur in most crocodilians and likely affects parental response to distress calls (Hutton, 1989). *C. porosus* females have been reported to stay with hatchlings until juveniles naturally disperse, usually after two months (Webb *et al.,* 1977; Bustard & Choudhury, 1980). In *C. rhombifer*, hatchlings have been reported to stay with their mother for up to six months and in *C. siamensis* this is predicted to be up to a year (Conners, 2002; Bezuijen *et al.,* 2012; Sam *et al.,* 2015). In *P. palpebrosus*, hatchlings remain together for up to 21 months, but extended care is predominantly fulfilled by the females (Campos *et al.,* 2012).

There is little information available for parental care in *T. schlegelii*. This is in part, due to low population numbers both in the wild and captivity (Fig. 1.1). Fear of humans could also explain why parental care has not been widely observed. *T. schlegelii* are considered relatively harmless (Hassan *et al.,* 2016), so a human presence likely triggers the flight response which causes the abandonment of nests and young. There have been few accounts of apparent nest guarding by females, who fled upon approach (Foster, 2013). Furthermore, *T. schlegelii* juveniles emit distress calls which indicates that extended care is present, as the main function of this call appears to be aid recruitment (Bonke *et al.*, 2015). Despite this, the lack of reported occurrences has led to the common belief that extended care is absent in this species (Britton, 2009; Vitt & Caldwell, 2009).

Fig. 1.1. *Tomistoma schlegelii* juvenile representing the second successful breeding of the species in Europe (Crocodiles of the World UK, 2017).

There have been reports of extended bi-parental care in *C. niloticus*, *C. siamensis* and *C. rhombifer* (Conners, 2002; Tullberg *et al.,* 2002; Sam *et al.,* 2015). In *C. niloticus* bi-parental care has been observed during the first few days post-hatching when parents, particularly females, are very responsive to distress calls (Hutton, 1989; Fergusson, 2010). In captive *C. siamensis*, both parents were observed defending their nests and hatchlings (Bezuijen *et al.*, 2012; Sam *et al.,* 2015). Intentional feeding of young has also been reported in captive *C. siamensis* (Whitaker, 2007). However, incidents of extended care from *C. siamensis* in the wild has only been observed in females (Bezuijen *et al.,* 2012; Sam *et al.,* 2015). A report on *C. rhombifer* in captivity observed biparental care, but care was predominantly fulfilled by the female (Conners, 2002).

1.2 Crocodilian Acoustic Communication

Crocodilians use a wide repertoire of vocalisations to convey a diversity of meaning. Adult crocodilians emit hisses, bellows and infrasound, whilst juveniles have a unique set of calls to communicate hatching, desire for group cohesion and distress (Herzog & Burghardt, 1977; Pough *et al.,* 2001; Vergne *et al.,* 2009). A large proportion of extended care observed in crocodilians is specifically elicited by juvenile distress calls (Chabert *et al.,* 2015).

The importance of auditory signals within crocodilian communication systems sets them apart from other reptiles (Pough *et al.,* 2001). Crocodilians can produce low frequency sounds below human hearing and can emit airborne and waterborne vibrations simultaneously (Pough *et al.,* 2001). The structure, timing and frequency of vocalisations appear to vary between species, with alligators most likely to vocalise out of the three subfamilies (Pough *et al.,* 2001). Additionally, crocodiles and caimans

share the same acoustic code in their vocalisations with evidence that different species calls can generate the same response (Mathevon *et al.,* 2013, 2016).

Crocodilian vocalisations have been previously placed into three categories; low frequency threatening sounds, high frequency distress calls, and 'interest calls', which are low in frequency but longer in duration (Brazaitis & Watanabe, 2011). Crocodilians have been observed to respond to these vocalisations by orientating or moving towards the sound source (Brazaitis & Watanabe, 2011). Vocalisations have been observed during courtship rituals, territorial displays and parental-offspring interactions (Pough *et al.,* 2001; Vergne *et al.,* 2009; Brazaitis & Watanabe, 2011). Juveniles vocalise to synchronise hatching (Vergne & Mathevon, 2008), maintain group cohesion (Vergne *et al.,* 2009) and to alert conspecifics to the presence of predators (Vergne *et al.*, 2007, 2011). Despite crocodilians' rich vocal repertoire, there has been relatively little research into the properties and behaviours that characterise their calls, particularly juvenile distress calls (Vergne *et al.,* 2009, 2011).

1.3 The Nature of Distress

Distress signals are utilised by plethora of species and can be emitted in an auditory, chemical or visual format (Fenton *et al.,* 1976; Staton, 1978; Hauser, 1986; Poel *et al.,* 1989; Knowlton *et al.,* 1999). Research into strategies of predation aversion in reptiles is often biased towards vision, with little knowledge on alternate mechanisms such as chemosensory, mechanosensory and audio sensory (Pough *et al.,* 2001). This bias needs to be addressed in order to fully understand predator-prey interactions. It is possible that multiple signals are emitted when crocodilian juveniles meet a predator, for example fin flicking in glowlight tetras (*Hemigrammus erythrozonus*) after distress pheromones are secreted (Brown *et al.,* 1999). The focus of this paper is on auditory signals. However, it is important to acknowledge additional channels of communication that may be utilised to signal distress.

Crocodilian eyes are uniquely adapted to visualize their environment both above and below the water surface. They possess a foveal streak, which is designed to provide maximum clarity when scanning across their visual horizon (Fig. 1.2; Nagloo *et al.,* 2016). Crocodilians use visual displays, such as head slapping and body posturing, to communicate during courtship and territorial claims (Kofron, 1991; Pough *et al.,* 2001). Visual distress signals have been observed in many aquatic species. Sixty-five species of batrachoidid fish use bioluminescent displays to signal distress in response to predators (Smith, 1992). However, there have been no reports of crocodilians using visual signals to communicate distress. This is probably because these signals are not as effective in habitats where visibility is obscured. This is supported by the findings that crocodilians living in open areas are less dependent on vocal signals than those (often alligator species), living in heavily vegetated habitats (Lang, 1989; Pough *et al.,* 2001; Murphy *et al.,* 2016;). Consequently, as crocodilians commonly inhabit heavily vegetated

and marshy territories, visual signals are unlikely to be as efficient at reducing predation rates as vocalisations (Pough *et al.,* 2001).

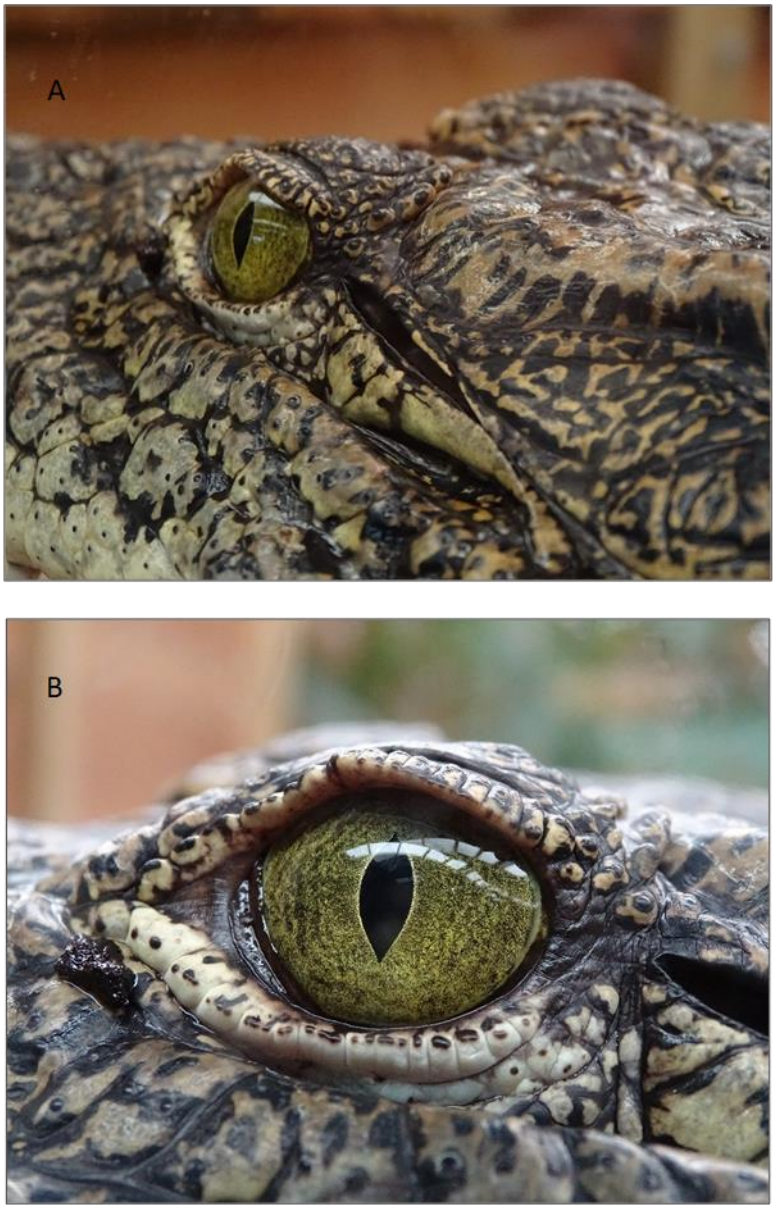


Fig. 1.2. Lateral view of *Crocodylus niloticus* head (A) and close-up of eye (B). Images show the crocodilian's foveal streak which is uniquely designed to scan movement across the visual field, both above and below the water surface (Nagloo *et al.,* 2016).

Chemical signals can have a significant effect on predator-prey interactions and are utilised by both aquatic organisms and reptiles (Madison, 1977; Pough *et al.,* 2001; Lönnstedt & McCormick, 2015). Many organisms release pheromones to elicit an antipredator response. For example, damselfish and fathead minnows (*Pimephales promelas*) secrete a chemical signal when they are seized by a predator (Chivers *et al.,* 1996; Lönnstedt & McCormick, 2015). This chemical signal attracts secondary

predators, which can disrupt the attack and increase chances of escape. Crocodilians possess a mandibular gland that become active (evert) during courtship displays and stress. Chemical signals are secreted from this gland which can be detected by conspecifics (Johnsen & Wellington, 1982; Weldon & Sampson, 1988; Brazaitis & Watanabe, 2011). Juvenile crocodiles may release chemical signals from these glands when distressed. Molecular analysis has revealed that the melanin-pigmented areas on almost all crocodylid and gharial scales, known as integumentary organs (ISOs), can detect chemical, thermal and mechanical changes within the environment (Di-Poï & Milinkovitch, 2013). Additionally, evidence of specialised organs on the tongue and posterior palate of *Alligator mississippiensis, C. niloticus* and *Crocodylus moreletti* suggest that they can detect chemical cues in the water (Fig. 1.3) (Weldon *et al.,* 1990; Platt *et al.,* 2006; Di-Poï & Milinkovitch, 2013). Male *C. niloticus* have even been observed touching the female's mandibular gland during courtship displays (Kofron, 1991). Therefore, the use of chemical signals in crocodilian communication is likely to occur. However, there is currently no evidence that chemical signals are capable of transmitting information about the individual's size. Therefore, vocalisations may be a more appropriate distress signal as receivers can adapt their response based on the encoded size information (Chabert *et al.,* 2015). Adapting care based on juvenile size is optimal as predation on crocodilians is largely size dependant (Somaweera *et al.,* 2013). This does not omit the possibility of chemical emittance in distressed juveniles.


A


B

Fig. 1.3. Evidence of integumentary organs (ISOs), concentrated areas of receptors, which can detect multi-sensory cues. ISOs have been discovered in the melanin-pigmented areas (which gives the appearance of dark spots) on the tongue (A), along the upper jaw (B) and dorsal scales (C) of *C. niloticus* (Di-Poï & Milinkovitch, 2013). Due to their heavily armoured skin, almost all crocodylid and gharial scales require ISOs in order to perceive touch and vibration.

Despite their heavily armoured skin (Fig. 1.3), crocodilians can detect both touch and vibrations due to the ISOs located on the surface of their scales (Leitch & Catania, 2012). The discovery of a predator often triggers the rapid movement of individuals in the water (Staton, 1978). This sudden movement produces vibrations which crocodilians can detect within a shared water body and then respond to. Therefore, it is possible that crocodilians can detect distress through mechano-sensory channels. Research into mechanosensory communication among crocodilians is relatively new, but it provides an interesting area of future work.

1.4 Distress Calls

When confronted by a predator a wide range of animal taxa emit acoustic distress signals (Aubin, 1991; Russ *et al.,* 1998; Vergne *et al.,* 2009; Eckenwebber & Knörnschild, 2016). There is a wealth of predators that prey upon juvenile crocodiles, including lizards, snakes, birds, turtles, larger crocodiles and on occasion, big cats (Somaweera *et al.*, 2013). When seized or startled by a predator, juvenile crocodilians transmit vocalisations (Herzog & Burghadt, 1977; Staton, 1978; Chabert *et al.*, 2015), which alerts conspecifics to the potential danger (Vergne *et al.*, 2011; Chabert *et al.*, 2015). Distress calls can

elicit protective behaviour from conspecifics (Pough *et al.*, 2001; Vergne *et al.*, 2011) and can also be directed at the predator itself (Conover, 1994).

Distress calls are often characterised by their repetitiveness and changing frequency as these are qualities that are easily located in space by the vertebrate ear, making them an efficient and fast form of communication (Marler 1955, 1961; Herzog & Burghadt, 1977). Crocodilian juvenile distress calls are equally characterised by short and rapid bursts of noise (Vergne *et al.*, 2008; Chabert *et al.*, 2015). Due to shared acoustic traits, distress calls often elicit interspecific responses (Aubin, 1991; Chabert *et al.*, 2015; Mathevon *et al.*, 2016). Adult crocodilians, both in the wild and captivity, have been observed to exhibit aggressive behaviour when played a distress call (Chabert *et al.*, 2015). There is experimental evidence that the playback of juvenile distress calls can indeed be used to attract adult crocodilians and can incite inter-specific responses (Staton, 1978; Chabert *et al.*, 2015; Mathevon *et al.*, 2016).

1.5 Functions of Distress Calls

There are a multitude of functionalities that distress calls can serve, aside from eliciting aid (Conover, 1994). To fully understand crocodilian behaviour, current hypotheses that aim to explain the functions of distress calls need to be explored.

1.5.1 Requesting aid

Distress call emittance can elicit an anti-predator response in conspecifics. This can disrupt the attack and increase the emitter's chance of survival (Rowher *et al.,* 1976). Most birds and bat species engage in mobbing behaviour when a conspecific emits a distress call (Stefanski & Falls, 1972; Russ *et al.,* 1998; Branch & Freeberg, 2012; Eckenwebber & Knörnschild, 2016). In crocodilians, requesting aid from a larger individual appears to be the primary function of juvenile distress calls (Staton, 1978; Pough *et al.*, 2001; Whitaker, 2007). This strategy increases the survival rate of juveniles at a relatively low cost to the responder (Lang, 1989; Staton, 1978). However, evidence that distress calls attract aid does not rule out the possibility of additional functionalities. For example, in addition to requesting aid, tufted titmice (*Baeolophus bicolor*) distress calls are reported to startle predators and to warn conspecifics of danger (Branch & Freeberg, 2012).

1.5.2 Warning conspecifics

In many group-living species, distress calls are used to warn nearby conspecifics of the presence and nature of a predator (Seyfarth *et al.,* 1980; Hogstedt, 1983). Vervet monkeys (*Chlorocebus pygerythrus*) use different calls to inform troop-members of an aerial or terrestrial predator (Seyfarth *et al.,* 1980). In

both crocodilians and *C. pygerythrus*, distress call emittance often results in the net movement of juveniles away from the sound (Staton, 1978).

Juvenile crocodilians are gregarious, they group together with other hatchlings that are not always kin (Lang, 1987). This behaviour has been observed in *C. porosus*, a species which is highly territorial and largely intolerant of conspecifics (Lang, 1987; Semeniuk *et al.,* 2011). Despite this, hatchlings form nurseries of mixed kin groups which then disperse after two months (Webb *et al.,* 1977; Brien *et al.,* 2013). The benefits of forming mixed kin nurseries overrides predisposed intolerance, indicating that a distress call system would benefit the fitness of the whole group (Passek & Gillingham, 1999). Whether females continue to guard these mixed pods indiscriminately is unclear (Brien *et al.,* 2013). Trivers (1971) reported that one of the indirect benefits of alerting conspecifics was the prevention of predators specialising on their species and location. Consequently, distress calls in crocodilians could act as a warning to other juveniles, as well as being a form of requesting aid.

1.5.3 Startling the predator

A possible function of distress calls is to startle a predator and deter the attack (Driver & Humphries, 1969; Conover, 1994; Wise *et al.,* 1999,). The startle response of avian distress calls reportedly works to an extent, on naive and inexperienced coyotes (*Canis latrans*) (Wise *et al.*, 1999). However, the population of *C. latrans* was fast to habituate which reduced call effectiveness. Passerine distress calls have also been observed to startle predatory opossums and racoons, and in some cases lead to the release of prey (Conover, 1994). Juvenile crocodilians commonly emit distress calls when seized by a predator; therefore, the calls could function to startle attackers (Staton, 1978). So far, no in-depth research has been conducted on the effects of crocodilian distress calls on predator success rates.

1.5.4 Attracting further predators

It has been observed that distress calls can attract additional predators that disrupt the initial attack and increase the chances of evasion (Hogstedt, 1983). Acorn woodpeckers use distress calls to attract secondary predators - large mammals that will distract the attacker - allowing the caller a chance of escape (Koenig, 1991). Additionally, some species of fish use chemical distress signals to attract secondary predators (Chivers *et al.,* 1996; Lönnstedt & McCormick, 2015). Juvenile crocodilians emit distress calls when seized by a predator. These calls can attract mature crocodilians that are unrelated or of a different species (Chabert *et al.,* 2015). Larger, non- related crocodilians often cannibalise juveniles and can be considered secondary predators. Additionally, as predation is largely size determined, what may predate on a juvenile could become prey for an adult crocodilian (Somaweera *et al.,* 2013). Therefore, responding to a distress call could be an advantage to both emitter and receiver. Attracting

secondary predators may be an additional function of crocodilian distress calls and therefore a shared trait among archosaurs.

1.5.5 An honest signal of fitness

It has been theorised that distress calls can honestly signal an individual's condition (Laiolo *et al.,* 2004). For example, it was found that lesser short-toed larks (*Calandrella rufescens)* with higher fitness emitted harsher distress calls (Laiolo *et al.*, 2004). This honest signal could deter predators as it indicates the likelihood of prey escaping. This information can benefit both parties as energy is not wasted on a failed attack. In crocodilians, variance in fundamental frequency and weak individual signature appear to be the only changing factors of distress calls (Bonke *et al.,* 2015; Chabert *et al.*, 2015). Additionally, gharial distress call duration is positively correlated to body size (Bonke e*t al.,* 2015). Therefore, call duration or fundamental pitch may be used by predators to indicate the prey's fitness (body size). However, juveniles continue to emit distress calls when seized by a predator, after a decision to attack has already been made.

1.6 Body Size and Distress Calls

The risk of predation in juvenile crocodilians is largely dependent on the size of the juvenile, with mortality rate and body length negatively correlated (Somaweera *et al.*, 2013). This is particularly obvious in sympatric living species, with the larger of the two crocodilian species often dominating in population density when there is an absence of human hunting (Somaweera *et al.,* 2013). It has been theorised that as crocodilians grow, they emit distress calls less frequently as natural predation rates decrease (Staton, 1978; Somaweera *et al.,* 2013). For example, *C. rhombifer* cease to emit distress calls from around two years of age (Murphy *et al.*, 2016).

The fundamental frequency of a juvenile distress call correlates to body size, with a lower dominant frequency indicating a larger juvenile (Bonke *et al.,* 2015; Chabert *et al.*, 2015). This is due to the palatal valve and resonators associated with vocalisation growing with body size, which makes them capable of producing lower pitched sounds (Vergne *et al.,* 2009; Taylor & Reby, 2010). Research into the fundamental frequency of communicatory calls in mammals have found that they can be used as a reliable indicator of body size (Pfefferle & Fischer, 2006). Crocodilians have advanced hearing capabilities - equal to birds and mammals- and they can detect this change in frequency (Staton, 1978; Pough *et al.,* 2001; Chabert *et al.,* 2015). Previous research on female *C. niloticus, C. intermedius* and *Caiman crocodilus* reported that responses to distress calls are influenced by the caller's size, with a higher response rate for smaller individuals (Chabert *et al.,* 2015; Mathevon *et al.,* 2016). Predation rate in crocodilians is largely size dependant. Adapting protective behaviour based on an individual's size

enables optimisation of care towards the most vulnerable offspring (Somaweera *et al.,* 2013; Chabert *et al.,* 2015). Gleaning size information from auditory signals is a tactic employed throughout the animal kingdom and can benefit both the caller and the receiver. Many species use call features to indicate the size and relative fitness of an individual, which helps avoid unnecessary physical conflict over finite resources such as territory, food and mates (Arak, 1983; Simmons, 1988; Wagner, 1992). This strategy is difficult to cheat as pitch tends to be directly related to the size of the sound producing structures (Zahavi, 1977; Briton, 2001; Bonke *et al.,* 2015).

1.7 Identifying Kin

In numerous species of birds and mammals, parents can use acoustic cues to identify their offspring (Charrier *et al.*, 2001). It is often genetically beneficial for individuals to optimise care towards related young (Hamilton, 1964). In *A. mississippiensis*, the mother moves her offspring to a nursery area near the nest site. Due to the distance between nest sites, young are likely to be her own and a kin recognition system would appear redundant (Passek & Gillingham, 1999).

However, in some species mixing of clutches occurs (Woodward *et al.,* 1984). *C. niloticus* can live in dense groups which amass more than 200 individuals (Lang, 1987). Females have been reported to form nurseries when there are many nests in the same area (Pooley & Gans, 1976; Pough *et al.,* 2001). Therefore, parents are exposed to the distress calls of non-kin. However, a recent study on 0–4 days-old juveniles found that calls are poorly individualized, making acoustic recognition of hatchlings improbable (Bonke *et al.,* 2015). An individual call-based recognition system in *C. niloticus* is therefore unlikely (Vergne *et al.,* 2006). Bird distress calls also contain few and weak individual signatures compared to their other acoustic signals (Charrier *et al.,* 2001). This could indicate certain benefits of responding to the distress calls of non-kin. Location of call may be a determinant of whether to respond. If the call is emitted within a territory, then there is an increased risk of predation for all vulnerable individuals. There is a higher frequency of mobbing behaviour in bats when distress calls are emitted near nesting sites as opposed to foraging sites (Eckenwebber & Knörnschild, 2016). Therefore, proximity to nest site could override the benefits of a kin recognition system within distress calls. Vergne *et al.,* (2007) proposed alternative communicatory channels that may contain individual signatures; such as chemical, olfactory or visual. Alternatively, collective caregiving may be present, which would explain why there are weak individual signatures between kin. Many crocodilian species have been reported to cooperatively hunt; they are likely to be neurologically capable of cooperative care too (Dinets, 2015b). It should be noted that individual recognition systems have not been researched in all species. Thus, strong individual signatures may be present in the distress calls of other crocodilian species.

1.7.1 Sex and care

It was predicted that female crocodilians would be more receptive to distress calls compared to males. This is because crocodilian females have been widely documented as the primary carer (Staton, 1978). The behavioural response of male crocodilians to distress calls is largely unknown as they have been relatively neglected in previous research (Chabert *et al.,* 2015; Mathevon *et al.,* 2016). In general, females are often the primary care giver of young because they have a high maternal certainty that offspring are genetically theirs, whereas paternal certainty is influenced by mating strategies and sperm competition (Hauser, 1986). Additionally, females have a higher initial investment in young as egg production is more energetically costly than sperm. Consequently, it was hypothesised that male crocodilians will be less responsive and slower to react to distress calls compared to the females.

1.8 Research Questions

1.8.1 Breeding period

Crocodilians were predicted to be more responsive to calls during their breeding period, as this is when young are present and at most risk from predation. Female crocodilians have a sudden heightening of aggression after the egg laying period which would be likely to increase response rate to distress calls (Brazaitis & Watanabe, 2011). During nesting, male *O. tetraspis*, *C. niloticus* and *A. mississippiensis* have lower levels of testosterone in their blood plasma which is then followed by a spike in the following two months (Kofron & Steiner, 1994). Therefore, male response rate to distress calls may also significantly alter during the nesting period. It was hypothesised that response rate would be on average higher and reaction time shorter during the breeding period.

1.8.2 Size of juvenile caller

It was predicted that receptiveness to distress calls will significantly change depending on the caller's size and that the smaller the juvenile caller the stronger the behavioural response that will be provoked. This correlation was discovered in other crocodilian species such as *C. niloticus* (Chabert *et al.,* 2015). Crocodilian distress calls contain strong information about the emitters size, with fundamental frequency as the core indicator (Vergne *et al.,* 2007). It has been speculated that crocodilians and alligators follow the same general rule when coding size information in their distress calls, and receivers modulate their response based on this (Chabert *et al.,* 2015). Work by Mathevon (2016) and Chabert *et al.* (2015) on female *C. intermedius*, *C. niloticus* and *C. crocodilus* revealed a higher response rate to the distress calls of smaller young. Chabert *et al.* (2015) collected data on four species of crocodilian and formulated a linear regression to demonstrate the correlation between fundamental frequency and size.

For this study the fundamental frequency of distress calls was manipulated according to the results from Chabert *et al.* (2015). Three different distress calls were created that matched a 20 cm, 60 cm and 160 cm juvenile. It was hypothesized that response rate would be highest for the 20 cm caller and lowest for the 160 cm caller and that average reaction time to distress calls would be quicker as juvenile size decreases.

1.8.3 Relative difference in body size between caller and receiver

It was predicted that the relatively small-bodied sized individuals within this sample will be significantly less responsive to the distress calls of larger juveniles in comparison to the relatively large-bodied sized individuals. Interpreting the size of an individual from their distress call may be beneficial to receivers that are similar in length as it signals a predator that could threaten them too. Mature *O. tetraspis* and *P. palpebrosus* do not exceed 180 cm in length and the individuals used in the sample were approximately 130 cm in length. Additionally, the *C. niloticus* within this study were immature and ranged from 90 - 150 cm. These four populations were considered 'small' as individuals within this group were smaller than a 160 cm distress caller. As predation is largely size dependent in crocodilians, it may be beneficial for these individuals to either not respond or move away from the distress calls of a larger 160 cm individual. The large size individuals in this sample were the *C. rhombifer*, *T. schlegelii*, *C. porosus* and *C. siamensis*. These crocodilians have a lower risk of natural predation and are therefore less likely to be threatened by the distress calls of a larger juvenile. It was hypothesised that the large bodied group would have a higher response rate to the 160 cm long distress callers compared to the small-bodied group.

1.8.4 Kin discrimination

Most crocodilian species are allopatric so there is no pressure for distress call divergence between species (Mathevon *et al.,* 2013). Mathevon *et al.* (2013) reported that crocodilians do not distinguish between own distress calls and those of other species. Additionally, distress calls contain weak individual signatures meaning differentiation between own young is unlikely (Chabert *et al.*, 2015). To test this, the *C. siamensis* population were either played *C. niloticus* or their offspring's distress calls, and their response rate was compared. The *P. palpebrosus* population were played distress calls of their own species belonging to either their offspring or unrelated young of the same species. It was expected that there will be no significant difference in responses. As a control, the *O. tetraspis* population were played the distress calls of *C. niloticus* juveniles and the distress calls of non-related juveniles from the *O. tetraspis* species. It was hypothesised that there will be no difference between response rate to related and non-related distress callers, nor between callers of the same or a different species, based on findings from reviewed literature that Archosauria distress calls contain weak individual signatures (Vergne *et al.,* 2007).

1.8.5 Extended care in *T. schlegelii*

Despite limited literature, there appears to be behavioural differences in care giving strategies amongst crocodilians species (Pough *et al.,* 2001). It is predicted that some species will respond more frequently to distress calls than others. Little to no signs of parental care have been reported in *T. schlegelii*, so it was expected that the *T. schlegelii* population would have a significantly lower response rate to distress calls compared to the other sample species. Additionally, as evidence of care appears weak or absent in this species, it was predicted that there would be no difference in response rate during the breeding and non-breeding period.

1.8.6 Socialness of breeding strategy

Crocodilians can form complex social structures. The extent of these structures appears to vary between species, with some more tolerant of conspecifics than others (Ross, 1998). A species was considered social if there were existing reports of gregarious nesting and juveniles forming mixed kin groups. It was predicted that the relatively social breeders would be more responsive to distress calls, as mixing of young indicates a higher likelihood of co-operative care. Therefore, even when young are not their own, individuals of social species may have a higher response rate to distress calls compared to the non-social breeders. For this study *P. palpebrosus*, *C. niloticus* and *C. porosus* were categorised as social breeders. These species tend to be more gregarious during nesting and early hatchling growth and Alligators have higher reported incidences of social play (Ross, 1998; Dinets, 2015a). The two *O. tetraspis* populations and *T. schlegelii* were classed as non-social breeders. In *T. schlegelii* there has been evidence of solitary

nesting and suggestions that mating suppression occurs in females living in proximity and *O. tetraspis* are known to be widely solitary (Bezuijen *et al.*, 1997; Mathew *et al.*, 2011). There was a lack of reported evidence for *C. siamensis* and *C. rhombifer* regarding their social breeding nature, so these populations were omitted from the analysis. It was hypothesised that the relatively social breeders would have, on average, a higher response strength to distress calls compared to the non-social breeders.

1.8.7 Summary of Aims

The first aim was to measure and compare the reaction times and response rates of the breeding populations prior to and during their breeding period. It was predicted that crocodilians will be more responsive to juvenile distress calls during their breeding period. There was a special interest in the *T. schlegelii* population as evidence of parental care in this species has yet to be scientifically investigated. It was hypothesised that response rate would be on average higher and reaction time shorter during the breeding period. However, for the *T. schlegelii* population, due to lack of evidence that parental care occurs in this species, it was predicted that there would be no difference in responses regarding breeding period.

The second objective was to investigate whether females were more responsive to distress calls than males by comparing the reaction times and response rates of the two sexes within the sample. It was hypothesised that male crocodilians will be less responsive and slower to react to distress calls compared to females.

The third aim was to see if the body length of the caller would trigger a difference in the response rates and reaction times of the sample populations. The distress calls of 20 cm, 60 cm and 160 cm long juveniles were played to the populations. It was hypothesized that response rate would be highest for the 20 cm caller and lowest for the 160 cm caller and that average reaction time to distress calls would be quicker as juvenile size decreases.

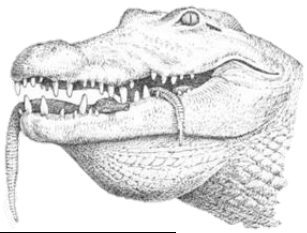
An additional aim was to test whether the relative difference in body size to the distress caller would affect responses. It was predicted that there would be differences in average response between the larger bodied crocodilians compared to the smaller bodied crocodilians within the sample. This was tested by categorising the sample populations into either large or small bodied groups and then comparing response rates and reaction times. It was predicted that the large bodied group would have a higher response rate to the 160 cm long distress callers compared to the small-bodied group.

Another objective of this study was to investigate whether crocodilians are more responsive to the calls of their own young as opposed to the calls of unrelated juveniles. The *C. siamensis* and *P. palpebrosus* populations were played distress calls belonging to their own young and unrelated juveniles, response

rates were then compared. It was hypothesised that there will be no difference between response rate to related and non-related young, nor between the same and different species distress calls.

Finally, it was predicted that the socialness of a species breeding strategy will influence response to distress calls. The sample populations were divided into either social or non-social breeding groups, depending on existing research (or excluded if no conclusive literature were found regarding their socialness). The responses were then compared. It was predicted that the relatively social breeders would have, on average, a higher response strength to distress calls compared to the non-social breeders.

2. Methods



2.1 Study Species

The playback sessions were conducted in two public zoos within the United Kingdom; Crocodiles of the World in Oxford and Bristol Zoological Gardens. Permission to conduct this experimental study was granted by the appropriate bodies from both Bristol Zoological Gardens and Crocodiles of the World. Overall, seven species were used in this sample, and 397 data points were collected. The sample consisted of *Crocodylus rhombifer*, *Paleosuchus palpebrosus, Crocodylus porosus*, *Tomistoma schlegelii*, *Crocodylus siamensis*, *Crocodylus niloticus* and *Osteolaemus tetraspis*, all sampled from Crocodiles of the World, Oxford. An additional *O. tetraspis* pair was sampled from Bristol Zoological Gardens. Excluding the *C. niloticus*, all individuals were sexually mature (Table 2.1). The *T. schlegelii*, *C. siamensis* and *O. tetraspis* populations were actively breeding at the time of study. This was a longitudinal study; the first playback session was undertaken on 12.12.2017 and the last on the 28.09.2018. The crocodilians were captive living and 'on-show' to the public, as a result they were habituated to a variety of audio and visual stimuli that would be abnormal in the wild.

Crocodiles of the World was comprised of two separate hangars. Hangar A contained the *O. tetraspis* and the *P. palpebrosus*. Hangar B housed the *C. rhombifer*, *C. siamensis*, *C. porosus*, *C. niloticus* and *T. schlegelii*. The Bristol population of *O. tetraspis* inhabited the zoo's reptile house and were the only crocodilian species present. Every breeding pair had access to nesting material, dry land and water. Environmental temperatures were artificially elevated to mimic conditions in the wild. All crocodilians were in good health and keepers maintained the highest standard of welfare. Permission to conduct these experiments was granted from both zoos. The playback experiments were non-invasive, and crocodilians remained in their enclosures. Initial playbacks were monitored by keepers to ensure that no severe distress was caused.

Table 2.1. Physical, social and reproductive characteristics of the sampled populations.

Species	Population Size	Population Sex	Breeding Status	Body Size Category	Social Breeders
Crocodylidae					
Osteolaemus tetraspis (Bristol Population)	2	Mixed	Active	Small	No
Osteolaemus tetraspis (Oxford Population)	2	Mixed	Active	Small	No
Crocodylus niloticus	34	Female	Non-active (sexually immature)	Small	Yes
Crocodylus siamensis	2	Mixed	Active	Large	NA
Crocodylus rhombifer	2	Female	Non-active	Large	NA
Crocodylus porosus	3	Female	Non-active	Large	Yes
Tomistoma schlegelii	2	Mixed	Active	Large	No
Alligatoridae					
Paleosuchus palpebrosus	5	Mixed	Non-active	Small	Yes

There are three families within the Order Crocodilian, these are Alligatoridae, Crocodylidae and Gavialidae. 24 extant species of crocodilians are currently recognised. Crocodilian distribution is wide, with populations throughout tropical, sub-tropical, temperate and wetland regions (Brazaitis & Watanabe, 2011). The following five sample species belong to the Family Crocodylidae. Species within this family can be further categorised into the *Crocodylus* or *Osteolaemus* genera (Pough *et al.,* 2001).

2.1.1 *Crocodylus porosus*

Crocodylus porosus (saltwater crocodile) is the largest extant crocodilian species, individuals can grow up to 7 m and have the widest global distribution (Pough *et al.,* 2001). *C. porosus* are classed as Least Concern (Crocodile Specialist Group, 1996a). The sample population was comprised of three females, each approximately 2.3-2.5 m long (Fig. 2.1). The females had not bred before but were of interest because this species reportedly forms mixed kin nurseries, despite normally being intolerant of conspecifics (Webb *et al.,*1977; Crocodile Specialist Group, 1996a; Brien *et al.,* 2013; IUCN, 2019).

Fig. 2.1. Two females from the sample population of *C. porosus*. Taken at Crocodiles of the World, Oxford.

2.1.2 *Osteolaemus tetraspis*

Osteolaemus tetraspis (West African dwarf crocodiles) are among the least understood crocodilian species (Shirley *et al.,* 2017). Originating from West and Central Africa, they are a relatively small species that tend not to exceed 1.8 m (Pough *et al.,* 2001; Franke *et al.,* 2013). *O. tetraspis* is classed as vulnerable on the IUCN red list (Crocodile Specialist Group, 1996b; IUCN, 2019). *O. tetraspis* commonly inhabit small streams in heavy canopy habitats (Magnusson *et al.,* 1990). However, a sub-population has recently been discovered living within a cave system in Gabon (Shirley *et al.,* 2017).

Two geographically disparate breeding pairs were used in this study. One pair resided at Bristol Zoo and the other at Crocodiles of the World, Oxford (Fig. 2.2). Molecular evidence can divide this species into four distinct lineages (Franke *et al.,* 2013; Shirley *et al.,* 2017). Molecular data available for the Bristol Zoo population categorised them as the Ogooué Basin form (Franke *et al.,* 2012). Both populations have had breeding success in the past. During this study the Bristol population laid a clutch of eggs in late June and the Oxford population laid a clutch in mid-May. The sampled individuals were approximately 1.3-1.4 m in length.

Fig. 2.2. Male *O. tetraspis* from the sample population at Crocodiles of the World, Oxford.

2.1.3 *Crocodylus niloticus*

Crocodylus niloticus (Nile crocodile) is a relatively social species, populations tend to occur in high densities (Pough *et al.,* 2001). *C. niloticus* are one of the largest crocodilian species. They can reach up to 6 m in length and are one of the most extensively studied crocodilian species (Fergusson, 2010). Population numbers are believed stable and are described as Least Concern (Isberg *et al.,* 2019). The *C. niloticus* population sampled for this study consisted of 34 sexually immature females (Fig. 2.3). Body length ranged from 1-1.6 m. This population was selected because *C. niloticus* responses to distress calls have not been researched in sexually immature individuals (Chabert *et al.,* 2016).

Fig. 2.3. Individuals from the *C. niloticus* population. Taken at Crocodiles of the World, Oxford. Gregarious basking (A) and Lateral view of head (B).

2.1.4 *Crocodylus siamensis*

Crocodylus siamensis (Siamese crocodile) is critically endangered with only fragmented populations scattered across South East Asia that are still breeding (Simpson & Bezuijen, 2010). Numbers continue to decrease due to habitat loss and illegal hunting (Bezuijen *et al.,* 2012). *C. siamensis* is a relatively large species. Males can reach up to 4 m in body length (Simpson & Bezuijen, 2010). Many captive populations have been hybridised with *C. porosus* and *C. rhombifer* to produce better quality skins for

farming (Fitzsimmons *et al.*, 2002). The sample population from Crocodiles of the World, Oxford was a genetically tested pure strain of *C. siamensis* (Crocodiles of the World, 2013). The sample consisted of a pair that had successfully bred in previous years and was actively breeding during the study period. The pair laid a clutch of eggs in mid-May (Table 2.1). The male was approximately 3 m (Fig. 2.4) and the female 2.3 m in length.

Fig. 2.4. Male *C. siamensis* basking. Taken at Crocodiles of the World, Oxford

2.1.5 *Crocodylus rhombifer*

Crocodylus rhombifer (Cuban Crocodile) are native to Cuba and are medium in size, with body lengths that do not exceed 3.5 m (Targarona *et al.,* 2010). The Crocodiles of the World (Oxford)population consisted of two females that were approximately 2 m long and had not bred before (Fig. 2.5). *C. rhombifer* were included in this sample because they are Critically Endangered and relatively little is known about their breeding behaviour (Targarona *et al.,* 2008).

Fig. 2.5. The population of *C. rhombifer* basking. Taken at Crocodiles of the World, Oxford.

2.1.6 *Paleosuchus palpebrosus*

Paleosuchus palpebrosus (Cuvier's Dwarf Caiman) are categorised within the Family Alligatoridae. They are found within forested areas in northern and central South America (Plough *et al.,* 2001). *P. palpebrosus* are a relatively small species. Individuals tend to grow to 1.8 m and numbers are reported as Least Concern (Campos *et al.,* 2010; Magnusson *et al.,* 2019). *P. palpebrosus* are nocturnal and largely feed on terrestrial vertebrates (Magnusson *et al.,* 1987; Pough *et al.*, 2001). The population from Crocodiles of the World, Oxford was a mixed sex group comprising of five individuals (Fig. 2.6). The group has had breeding success in the past but not during the study. Body lengths ranged from 1 - 1.3 m.

Fig. 2.6. Lateral view of *P. palpebrosus* head, taken from the sampled population in Crocodiles of the World, Oxford.

2.1.7 *Tomistoma schlegelii*

Tomistoma schlegelii (false gharial) classification is controversial because phylogenies have classed *T. schlegelii* into two different families; Gavialidae (molecular) and Crocodylidae (morphological) (Pough *et al.,* 2001). In the wild, *T. schlegelii* can be found in the freshwater streams of Malaya Peninsula, Sumatra and Borneo (Vitt & Caldwell, 2009). *T. schlegelii* can reach 4 m in length and have a distinctive elongated snout (Pough *et al.,* 2001; Vitt & Caldwell, 2009). Wild populations of *T. schlegelii* are small and information regarding their life history is limited. The number of mature individuals in the wild is estimated at 2,500 - 9,999 (Bezuijen *et al.,* 2014). The species is therefore classed as Vulnerable (Bezuijen *et al.,* 2014). *T. schlegelii* are rarely kept in captivity and have low breeding success. Consequently, observations of extended care are limited and widely considered non-existent (Bonke *et al.,* 2015). The pair from Crocodiles of the World, Oxford was actively breeding and thus offered a unique opportunity to study (Fig. 2.7). The pair had successfully bred in the previous year and laid another clutch during the study period in mid-May (Table 2.1). The male was approximately 3.6 m in length and the female was around 2.7 m. Very little is known about parental care in *T. schlegelii* and they are therefore a species of interest.

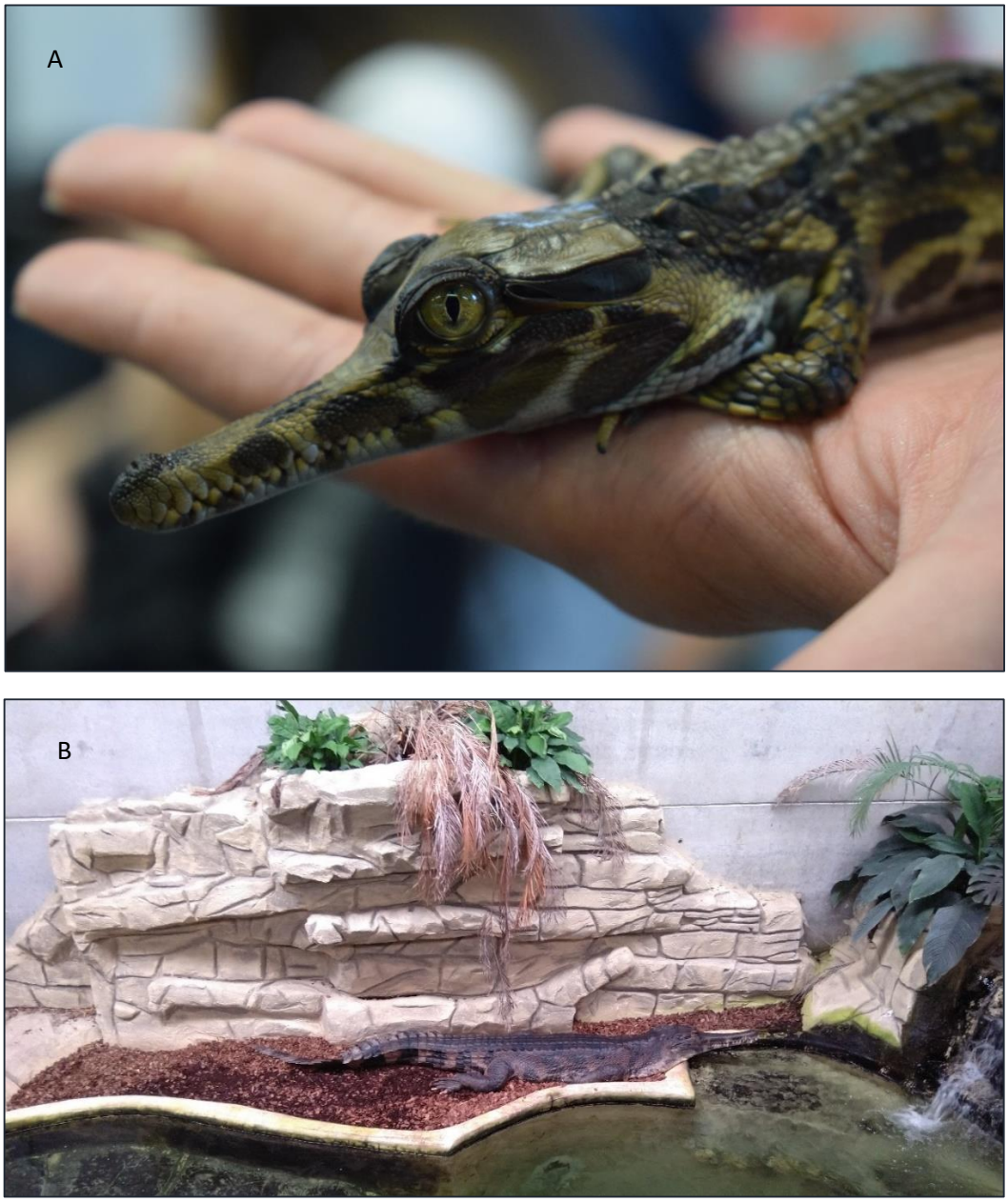


Fig. 2.7. (A) *T. schlegelii* hatchling from the sample populations' previous 2017 clutch. (B) *T. schlegelii* female basking. Taken at Crocodiles of the World, Oxford.

2.2 The Distress Calls

The distress calls of *C. rhombifer*, *C. porosus* and *P. palpebrosus* were collected from open access video-sharing platforms. The sampled juveniles' body lengths varied from 20 - 60 cm, measured from snout tip to tail end. The *C. rhombifer* distress calls came from a juvenile approximately 10 cm long. The *C. porosus* juvenile was approximately 60 cm and the *P. palpebrosus* juvenile was estimated to be 20 cm.

The *C. niloticus* distress calls belonged to a 35.5 cm juvenile and was sourced from Chabert *et al.* (2015). In some cases, the open-access footage did not provide information on the juvenile's size. Estimations were made by comparing the handler's palm size in the video (average human hand 189 mm) to the specimen. The control call came from a crying human baby and was also sourced from a video sharing platform (see Appendix).

The *C. siamensis* and the second set of *P. palpebrosus* calls were recorded from 2-year-old juveniles belonging to the sample population's offspring. These calls were recorded on site at the Crocodiles of the World Zoo on 06.07.2018 and body size was measured in the conventional way; from snout tip to tail end. The *C. siamensis* juvenile was measured at 55 cm and the *P. palpebrosus* juvenile at 45 cm. The juveniles were gently manipulated by a staff handler to induce distress calls. This was recorded with a digital recorder (R-05, Roland Corporation, Hamamatsu, Japan) device. The calls were used in playbacks sessions from 12.07.2018.

T. schlegelii distress calls could not be sourced due to the species' elusive nature and the young's natural reluctance to vocalise. *C. niloticus* distress calls were played to this species throughout the study. Species specific recognition based on juvenile calls has been documented as extremely weak or non-existent in crocodilians (Chabert *et al.,* 2015). Additionally, *T. schlegelii* distress calls have a similar structure to *C. niloticus* calls (Bonke *et al.,* 2015). There was no existing evidence that playing a different species call would cause a significant discrepancy in results.

The calls of the *C. niloticus* juveniles were also played to the *O. tetraspis* until the distress call of an *O. tetraspis* juvenile was used from 16.07.2018. The recording of the *O. tetraspis* distress call came from an 80 cm juvenile and was donated from field research in the Ivory Coast.

2.2.1 Creation of sequences

The distress calls were edited using the audio software SAS Lab pro (Avisoft Bioacoustics, Berlin Germany) to create a 60 s sequence which contained on average 30 individual calls. The individual calls within the sequences were on average 2 s apart (+/- 1 s) to reflect the natural emission pattern of juvenile distress signals (Vergne *et al.,* 2011; Chabert *et al.,* 2015, Fig. 2.8). The *C. siamensis* distress calls deviated from this as the natural sequence contained an average of five calls emitted within 0.6 s of each other, followed by an average 2 s break (Fig. 2.9). This pattern was retained in the modified sequences to preserve the temporal rhythm, which appears to be a defining acoustic characteristic in this species. Consequently, the modified sequence had an average 2 s break between each set of five calls. The resulting 60 s *C. siamensis* sequence consisted of 18 sets of these individual calls (Fig. 2.9).

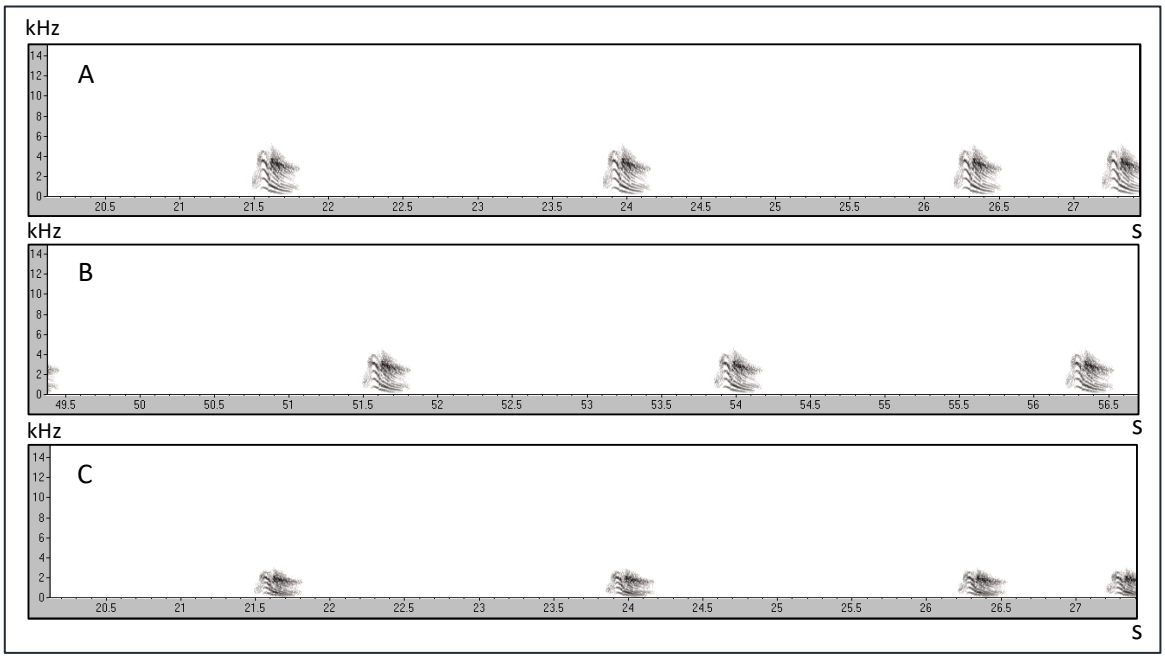


Fig. 2.8. Spectrograms of the three *C. niloticus* distress call sequences for each body length used in playback sessions (20 cm, 60 cm, 160 cm). (A) 20 cm juvenile distress caller mean pitch: 472.60 Hz. (B) 60 cm juvenile distress caller mean pitch: 413.80 Hz. (C) 160 cm juvenile caller mean pitch: 266.80 Hz. Spectrograms created with Avisoft SASLab Pro; FTT length: 512 points Hamming Window; 44.1 Hz sampling rate; 50% window overlap resulting in 86 Hz frequency resolution and 5.81ms temporal resolution.

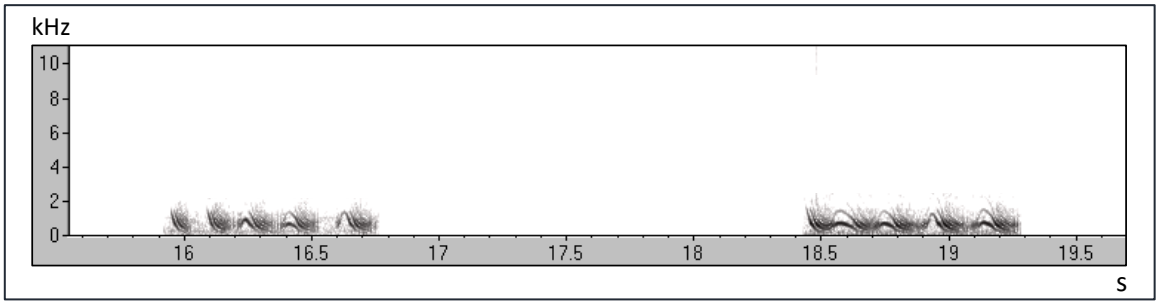


Fig. 2.9. Spectrogram of *C. siamensis* juvenile distress call (55 cm). Created with Avisoft SASLab Pro; FTT length: 512 points Hamming Window; 44.1 kHz sampling rate; 50% window overlap resulting in 86 Hz frequency resolution and 5.81 ms temporal resolution.

In juvenile crocodilians the pitch (Hz) of a distress call lowers as size increases (Chabert *et al.,* 2015). Chabert *et al.*, (2015) analysed the acoustic structure of distress calls emitted by 164 juveniles, using five species of crocodilians and outlined the formula that demonstrates this relationship between dominant fundamental frequency and body size. The formula for Crocodylidae species was: Y= -1.47x [size] + 502, and for species of alligatoridae Y = -2.46x + 658 (Chabert *et al.,* 2015). The equation was utilized in

this study to find the pitch difference between the original juvenile's distress call and the call of a juvenile with the desired body length of 20 cm, 60 cm or 160 cm. Pitch ratio was transformed into a percentage and this percentage change was implemented with the WavePad audio software. The software was used to modify the distress calls dominant fundamental frequency to three different juvenile sizes. The sizes were chosen to represent a small juvenile (20 cm), an intermediate sized juvenile (60 cm) and a large (160 cm) juvenile (Fig. 2.8). The acoustic software WavePad Audio Editor and Avisoft SAS Lab pro were used in this study to modify the pitch of the distress calls and to clean the calls of background noise (Fig. 2.8).

2.3 Data Collection

The study began in Bristol Zoo on 12.12.2017 with the *O. tetraspis* pair and ran until 29.10.2018. The first experimental session at Crocodiles of the World took place on 20.12.2017 and ran until 27.09.2018. Experimental times varied between 11:30-18:30 but were on average between 13:00-15:00. 21 sessions were conducted at Crocodiles of the World and 10 sessions at Bristol Zoo (Table 2.2). Initially, three sequences were played per session to each population, but due to habituation this was reduced to two sequences from playback session four. Playback sessions were on average two weeks apart. The size of the distress caller and the order that populations were tested was randomised for each session to minimise order effects. On average the populations were played two distress sequences within a playback session. In some cases, the recorded responses were invalid due to extraneous variables, such as visitor interference and thus were omitted from the results (N = 22). Habituation was taken into consideration and the order of each playback session was recorded and treated as a covariate during the statistical analysis.

Table 2.2 The frequency and distribution of the collected data from all the playback sessions according to the type of distress call sequence that was played (species and size (cm)). Tables (A-H) are divided by the sample populations that the data relates to.

A	*C. niloticus*	Audio Sequence	Size (cm)			
			20	60	160	Total
		C. niloticus	14	11	12	87

B	*C. porosus*	Audio Sequence	Size (cm)			
			20	60	160	Total
		C. porosus	18	18	10	86

C	*C. rhombifer*	Audio Sequence	Size (cm)			
			20	60	160	Total
		C. porosus	12	11	9	82

D	*C. siamensis*	Audio Sequence	Size (cm)			
			20	60	160	Total
		C. niloticus	20	18	14	52
		C. siamensis	55 (offspring)			16

E	*O. tetraspis* (Bristol Population)	Audio Sequence	Size (cm)			
			20	60	160	Total
		C. niloticus	12	14	14	40

F	*O. tetraspis* (Oxford Population)	Audio Sequence	Size (cm)			
			20	60	160	Total
		C. niloticus	18	20	19	57
		O. tetraspis	6	6	4	16

G	*P. palpebrosus*	Audio Sequence	Size (cm)			
			20	60	160	Total
		C. niloticus	10	8	12	80
		P. palpebrosus	45 (offspring)			8

H	*T. schlegelii*	Audio Sequence	Size (cm)			
			20	60	160	Total
		C. niloticus	27	22	24	78

2.3.1 Experimental setup

A loudspeaker (JBL model) was suspended from a corner of the enclosure and where possible, in an area obstructed from the crocodilians' view. This was lowered on average 30 cm from the ground and, if applicable onto the former nest site. The position of the speaker aimed to mimic the location of a juvenile. Effort was made to not alert individuals to the speaker's presence before the call sequence was initiated and thus maintain an appropriate level of ecological validity. Preliminary studies evaluated that approximately 30 cm from ground level allowed enough time to pull the speaker to safety if a crocodilian investigated the stimulus. Some individuals became increasingly aware of the speaker as the study progressed. In these cases, the speaker was readjusted to a higher position, moved to a new area of the enclosure, or hidden behind foliage. Any deviations in speaker location from the norm was noted. An action camera (YI 4K Sports Action Camera) was affixed below the speaker. This provided close proximity head orientation and displacement data (Fig. 2.10. A second camera (Nokia 6s) remained on the other side of the enclosure, offering a wide-angle view of outlying specimens. Both devices recorded audio which enabled footage to be later synced with the respective sequence that was played.

Fig. 2.10. Screen shots from video recorded with the YI 4K Sports Action Camera of *C. niloticus* investigating the source of a playback distress call. Taken from Crocodiles of the World, Oxford.

2.3.2 Playback sessions

Behaviour was recorded 10 min before and after the experimental procedure to ensure that any changes in behaviour were triggered by the distress sequences. After a preliminary period (three sessions), it was evaluated that, in order to minimise habituation, only two of the three size conditions would be tested per playback session. A minimum of 10 min latency time was given before playing the second sequence. Preliminary studies revealed that 10 min was adequate time for individuals to return to their pre-call behaviour. If a specimen had been displaced from its pre-call location, they often moved back to their original position within this time frame. Sequences were normalised to 75% and played at the same

volume from a HP Pavilion laptop that was connected wirelessly to a portable JBL speaker with a frequency range of 120Hz - 20000Hz. The speaker was attached via carabiner to a cable that allowed it to be lowered by hand. Sequences were not played until all specimens were stationary. Behavioural changes that occurred 5 s after the sequence had finished playing were not recorded as these were likely to have been caused by an alternate stimulus.

The order of the caller body size conditions that were played was randomised for each session. If individuals became aware of the speaker as it was lowered, then a further 6 min minimum wait was implemented before proceeding. In cases where the individual was still alert to the speaker, so it was moved to a new location. Incidences where speaker relocation failed to dissuade the crocodilians' attention the data for that playback session was omitted (N = 8). The aim was to test all sample populations per playback session. The order of playback sessions for the Crocodiles of the World sample was randomised for each visit to minimise the effect of calls on neighbouring populations. Control observations were conducted on non-target populations. From the control, it was concluded that individuals were unlikely to be affected by distress calls played to neighbouring enclosures (at an observable level). The 10-min latency time between playbacks would have helped to reduce these effects.

2.3.3 Measuring behaviour

Any changes to the target species' behaviour during sequence playback was recorded using the two video cameras and later analysed. Behaviours observed included displacement, inflation and deflation of stomach, head movement and orientation of body towards the sound source. If there was an observed change in the behaviour of the target population then this was recorded as the response rate. Additionally, if a response occurred, the strength (ethological scale) and time taken to respond (s) was also noted. This created three types of data; response rate (binary), reaction time (linear) and strength of response (ordinal).

Response Rate: For each session an observable behavioural change to a distress call was recorded as 1. No behavioural change was recorded as 0.

Reaction Time: The speed at which a response was initiated after a sequence began playing was measured to the nearest second. Reaction time data could only be collected when a response to a distress call had been elicited (N = 127).

Response Strength: Strength of response during playback sessions were ranked using an ethological scale from -1 to 2. 0 being no observable reaction to acoustic playback. 1 = slight response, such as head orientation towards sound source or visible changes in breathing/abdominal inflation. 2 = strong response, this category represented movement towards the speaker or indication that a protective behaviour has been initiated (Fig. 2.11). -1 = evasive response, this category represented movement away from the

distress signal or moving deeper underwater. If more than one individual responded from a population then the strongest response to occur was recorded, unless sex could be identified.

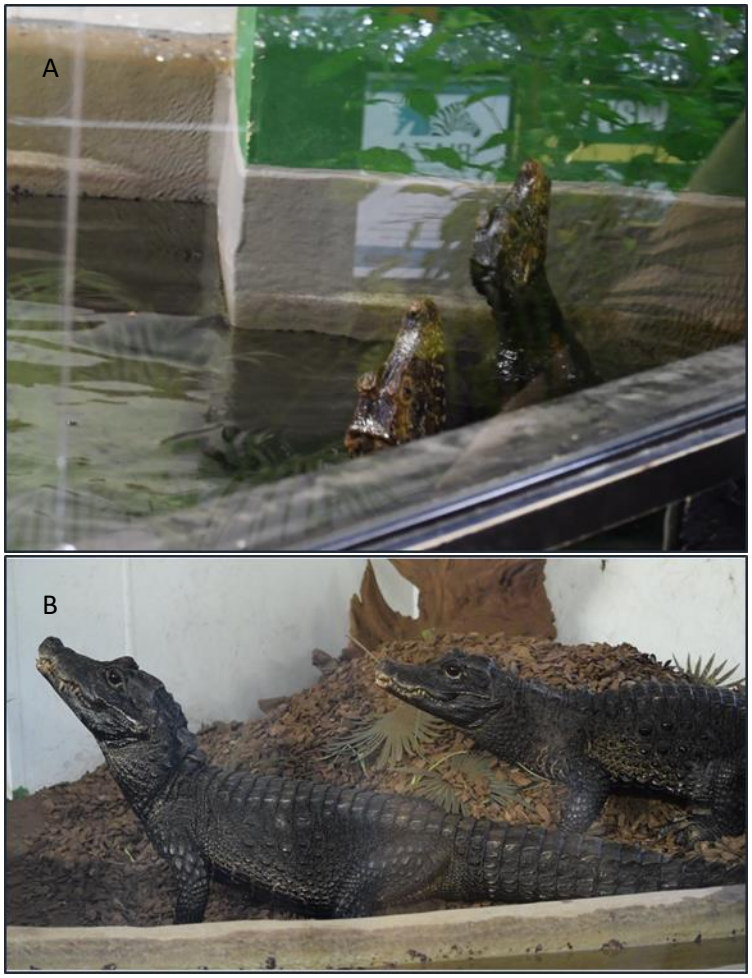


Fig. 2.11. Video screenshots of *P. palpebrosus* (A) and *O. tetraspis* (B) moving towards sound stimulus during distress call playback. Images illustrate a category '2' response on the ethological scale of response strength.

2.3.4 Pseudo-replication and habituation

Due to the limited sample size and time constraints on the experimental period, there was a risk that habituation and pseudo-replication would influence the results of this study. The following measures were taken to minimise these effects. Variables were randomised, such as the days in which playback sessions were conducted and the order in which sequences were played. This was to reduce the likelihood of individuals within the sample associating false distress calls with certain temporal patterns. Additionally, the observations and experimental setup were conducted as discretely as possible in order to not alert the

populations that a sequence was about to be played. To further reduce the effects of habituation there could have been a larger latency period between the field sessions (See Limitations). However, due to time limitations, conducting the sessions on average once every two weeks was necessary in order to collect enough data for the statistical analysis.

Due to sample limitations, only 6 populations (52 individuals) were studied. In order to obtain enough data, a repeated measures design was used. To measure the effects of habituation, the number of playbacks an individual was exposed to previously was treated as an extraneous variable. This was achieved by recording the number of sessions that had occurred prior to each playback session and any correlations within this was investigated. If playback session order was deemed significant by the statistical analysis, then this was reported alongside the results as evidence that habituation was likely to have influenced the results. However, even when no statistical significance was found, observations revealed that rapid habituation appeared to occur in all sample populations as there was an initial decrease in stimulus response during the first few playback sessions, which then plateaued.

The influence of pseudo-replication was mitigated by the use of the Generalised Estimating Equation (GEE). The GEE is one of the few statistical tests that accounts for autocorrelated data (See Statistical Analysis). Therefore, when individuals could be discerned from a population, the effects of pseudo-replication within the data could be largely accounted for. The findings from the GEE analysis was used to support the additional statistical tests employed by this study.

2.4 Testing the Predictor Variables

2.4.1 Breeding period

The breeding period encapsulated the egg laying period and after hatchlings had emerged, when young are at most risk of predation (Table 2.3). Each populations' breeding period was initially divided into three stages; egg laying, incubation and post hatching. There was no significant difference in response between the stages, so data was combined and then compared to the data collected during the non-breeding period. Nest building and egg laying for each species was monitored and recorded by keepers. The playback sessions began in the non-breeding period of each populations' reproductive cycle. The start of the breeding period varied between the two zoos and from what is expected in the wild. When eggs were not hatched approximated emergence was calculated using information from previous breeding years and from the wild. Only the *T. schlegelii* eggs were hatched.

Table 2.3. Dates for the stages within the breeding and non-breeding period for the actively breeding populations. ~ = Approximate date.

Sample Population	Pre-Laying Period	Egg Incubation Period	Post Hatching Period	Bred in previous year (2017)
C. siamensis	20.12.2017-10.05.2018	10.05.2018-25.07.2018~	~26.07.2018	Yes
O. tetraspis (Oxford Population)	20.12.2017-10.05.2018	10.05.2018-09.08.2018~	~10.08.2018	Yes
O. tetraspis (Bristol Population)	12.12.2017-29.06.2018	30.06.2018-29.09.2018~	~30.09.2018	Yes
T. schlegelii	20.12.2017-10.05.2018	10.05.2018-17.08.2018	17.08.2018	Yes

The breeding populations from Crocodiles of the World (Oxford) laid their eggs approximately on the 10.05.2018 (estimated accuracy +/- 1 weeks) which were discovered after keeper enclosure checks. The Bristol population laid on 30.06.2018. In cases where eggs did not hatch, the estimated incubation time was calculated from successful years and previous research. For *C. siamensis* populations in the wild, nesting occurs in the dry season towards April and February (Simpson & Bezuijen, 2010). Hatching then coincides in the wet season after an incubation period of 70 - 80 days, therefore the *C. siamensis* hatching date was predicted to have occurred by 26.07.18 (Simpson & Bezuijen, 2010). The *O. tetraspis* hatching date was approximated from the pair's previous incubation lengths, as 10.08.2018 for the Oxford population and 30.09.18 for the Bristol population. Interestingly, the *T. schlegelii* eggs were laid a month before the average female in captivity is expected to construct nests (June-July) (Pough *et al.,* 2001; Vitt & Caldwell, 2009). The *T. schlegelii* eggs began hatching on 17.08.2018.

2.4.2 Sex and care

The sex of the individual was recorded in each playback session for the *C. siamensis*, *O. tetraspis* and *T. schlegelii* populations as identification of sex was possible due to dimorphism (Fig. 2.12). The *P. palpebrosus* were the only other mixed sex group in the sample. The population displayed no obvious sexual dimorphism and therefore the individual's sex was not recorded.

Fig. 2.12. Male and female *C. siamensis*. The size dimorphism within the sample population of *C. siamensis* allowed for clear identification of sex (female with smaller body size on right side of image).

2.4.3 Size of juvenile caller

Size of distress caller was recorded for each playback session (20 cm, 60 cm and 160 cm) along with the order it was played within that session.

2.4.4 Relative difference in body size between caller and receiver

The sampled species (N = 7) were divided into two size categories based on body length (cm). This categorisation was important for the comparison tests used to assess the effects of relative size on response. *O. tetraspis*, *P. palpebrosus* and the sexually immature *C. niloticus* populations were categorised into the small body size group (100-160 cm) and *C. porosus*, *C. siamensis*, *C. rhombifer* and *T. schlegelii* were categorised into the large body size group (200-300 cm).

2.4.5 Kin discrimination

Recordings were taken from the distress calls of the *C. siamensis* and *P. palpebrosus* sample's offspring, hatched from the previous year and recorded on 12.07.2018. The recorded pitch was unmodified in the playback sequences to represent the size the juveniles were recorded at, 45 cm (*P. palpebrosus*) and 55 cm (*C. siamensis*). For the non-kin comparison, *C. siamensis* was exposed to the distress calls of *C. niloticus* and the *P. palpebrosus* population was exposed to non-related *P. palpebrosus* distress calls. The non-kin distress calls represented 20 cm, 60 cm and 160 cm sized juveniles. Response rates to the two caller conditions (kin and non-kin) were then compared.

The control group, *O. tetraspis* (Oxford population) was played own species calls between 26.07.2018 and 19.09.2018 and the *C. niloticus* distress calls prior to and after this period. Response rates to the two caller species were then compared.

2.4.6 Socialness of breeding strategy

From research it was determined that *C. niloticus*, *C. porosus* and *P. palpebrosus* were social breeders (see Introduction). The response rate and reaction time of the social breeders' group was compared to the results of the non-social breeder's group, which encompassed the *O. tetraspis* and *T. schlegelii.*

2.5 Statistical Analysis

The binomial logistic regression model (BLM), multi-linear regression (MLR) and Mann-Whitney U test all function with non-parametric data. Therefore, these tests were used to predict a population's response rate, response strength and reaction time based on the following independent variables; caller body size, sex, breeding period, number of playback sessions, socialness of breeding strategy, species, relatedness to caller and relative size. The disadvantage of using these models with auto-correlated data is the increased risk of false positives. To increase confidence that false positives had not occurred the output of these models were reported alongside the results of the generalised estimating equation (GEE). The GEE is a regression technique that can estimate the average response within a population. The advantage of using the GEE regression is that it does not assume independence of observations and works well with correlated data. However, unlike other regression models, the GEE cannot predict the effect of one or more variables on a population's response. Additionally, as the *C. rhombifer*, *C. porosus* and *C. niloticus* populations were all females and the *P. palpebrosus* showed no dimorphism, these populations could not be split into sub-groups. The limitation of this is that the GEE requires a between subject variable in order to run successfully. An outgroup was therefore needed. When comparing behavioural differences to caller body size in these populations, data points were randomly divided to create the outgroups (N= 2).

A binomial logistic regression model was run on the response rate data using the IBM SPSS Statistics 25 program. The binomial logistic regression tests for significance within a dichotomous dependent variable and predicts the probability of response to a distress call based on various categorial independent variables.

The Mann-Whitney U test was run on the response strength data using the IBM SPSS Statistics 25 program. Species' socialness and relative size to the distress callers were the predictor variables which

were investigated. The Mann-Whitney U test can determine differences between groups using an ordinal dependant variable and a dichotomous independent variable.

A multi-linear regression model was run on the reaction time data using the IBM SPSS Statistics 25 program. The multiple linear regression model is used for continuous dependent variables and predicts the effect of multiple independent variables. A regression equation was created from this analysis which can be used to predict the effects of different independent variables on reaction time (s).

3. Results

3.1 Multi-linear Regression Results (MLR)

The multi-linear regression (MLR) was run on the four breeding populations. There was homoscedasticity in the results, as assessed by a plot of studentized residuals versus unstandardized predicted values. The assumption of no multicollinearity in the data was met by an assessment of the tolerance values which were all greater than 0.1. There were nine leverage values greater than 0.2. However, there were no values for Cook's distance above one, so they were deemed not concerning. There were four data points that had standard residuals of more than +/- 0.3, these were removed from the test.

The MLR predicted reaction time (F (2,68) = 13.33, $P < 0.001$, adj. R2 = 0.26). The following two variables added statistical significance to the prediction, sex ($P < 0.001$) and breeding period ($P < 0.001$). Regression coefficients and standard errors are reported in Table 3.1 The predictor variable caller body size was not significant. The MLR predicted the following reaction time model equation for breeding populations as a function of season and sex:

$$13.81 - (4.87 \times \text{season}) - (5.83 \times \text{sex})$$

Note: male = 0 and female = 1, non-breeding period = 0 and breeding period = 1

Table 3.1. MLR output for reaction time with sex and breeding period as predictor variables. Note: B= unstandardized regression coefficient (non-breeding period and male are the intercepts). S.E.= standard error of the coefficient and β = standardized coefficient.

Variable	B	SE_B	β	*p*	95% C.I for B	
					Lower	Upper
Intercept	13.81	3.00	-	-	13.81	25.80
Sex	-5.83	1.56	-0.39	<0.001	-8.94	-2.72
Breeding Period	-4.87	1.32	-0.38	<0.001	-7.50	-2.23

3.2 Binomial Logistic Regressions Results (BLR)

3.2.1 Binomial logistic regression (BLR) for *P. palpebrosus*

The BLR model for *P. palpebrosus* was statistically significant, $\chi 2(3) = 8.04$, $P = 0.045$ (Table 3.2). Kin calls elicited a higher response rate (See 'Kin Discrimination'). The area under the Receiver Operating Characteristic (ROC) curve was 0.746 (95% C.I. = 0.590 - 0.902), an acceptable level of discrimination according to Hosmer *et al.* (2013) (Fig. 3.1).

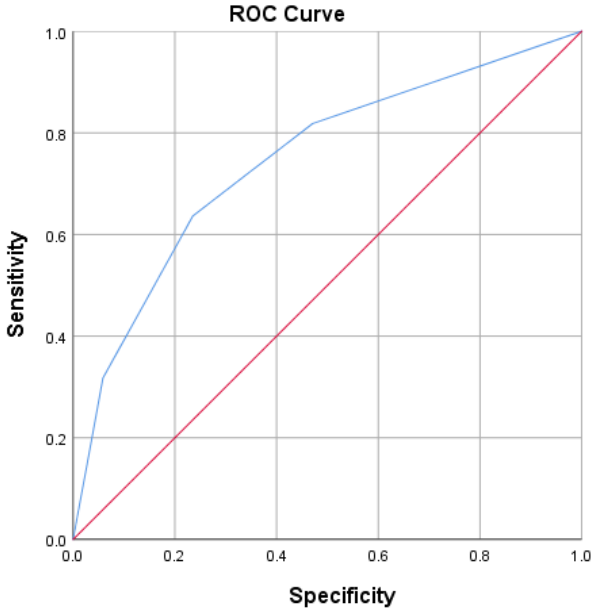


Fig. 3.1. ROC curve for *P. palpebrosus* response rate, with caller body size as a predictor variable.

Table 3.2. Binomial linear regression success at explaining variance for populations' response rates

Population	*p*	Variance Explained, Nagelkerke R^2 (%)	Cases Correctly classified (%)	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	Discrimination Level (Hosmer *et al.*, 2013)
P. palpebrosus	$\chi^2(3) = 8.04$, $p = 0.045$	25.0	69.2	81.8	52.9	69.2	69.2	Acceptable
T. schlegelii	$\chi^2(2) = 36.89$, $p < 0.001$	56.2	86.3	81.8	88.2	75.0	91.8	Excellent
C. siamensis	$\chi^2(5) = 35.99$, $p < 0.001$.	58.5	85.3	65.0	93.8	81.25	86.5	Excellent
O. tetraspis (Bristol)	$\chi2(2) = 8.38$, $p = 0.015$	25.4	67.5	88.2	52.2	57.6	85.7	Acceptable
O. tetraspis (Oxford)	$\chi2(2) = 17.24$, $p < 0.001$	35.9	84.7	50.0	91.7	54.5	90.2	Excellent

3.2.2 Binomial logistic regression (BLR) for *T. schlegelii*

The BLR model for *T. schlegelii* was statistically significant ($\chi2(2) = 36.89$, $P < 0.001$; Table 3.2). Two predictor variables were statistically significant: odds of response were higher in the breeding period (see 'Influence of Breeding Period') and for females (see 'Influence of Sex on Level of Care'). The area under the ROC curve was reported as 0.877 (95% C.I = 0.790 - 0.964). The area demonstrated an excellent level of discrimination (Hosmer *et al.,* 2013; Fig. 3.2). There were four standardized residuals that were above three standard deviations which were investigated and kept in the analysis.

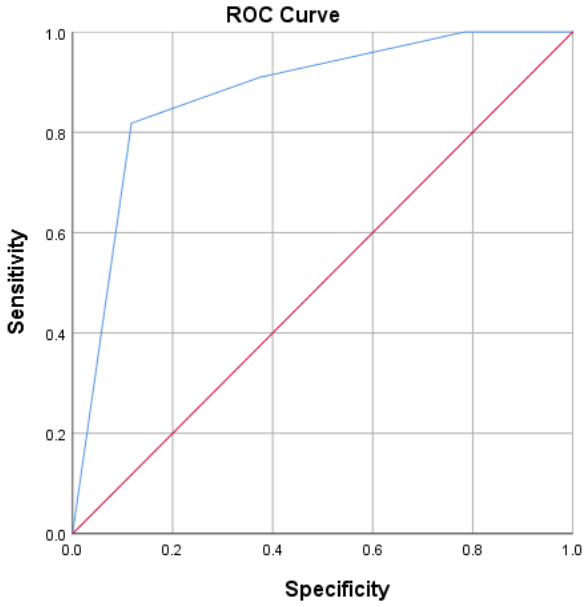


Fig. 3.2. ROC curve for *T. schlegelii* response rate, with breeding period and sex as predictor variables.

3.2.3 Binomial logistic regression (BLR) for *C. siamensis*

The BLR model for *C. siamensis* was statistically significant, ($\chi2$ (5) = 35.988, $P < 0.001$; Table 3.2). Two predictor variables added significance, sex (see 'Influence of Sex on Level of Care') and kin discrimination (See 'Kin Discrimination'). The female was more likely to respond over the male and playing their offspring's distress calls were more likely to elicit a response. There were two standardized residuals above the value of three standard deviations in the data that were investigated and kept. The area under the ROC curve was 0.893 (95% C.I. 0.813 - 0.974), which is an excellent level of discrimination according to Hosmer *et al.* (2013; Fig. 3.3).

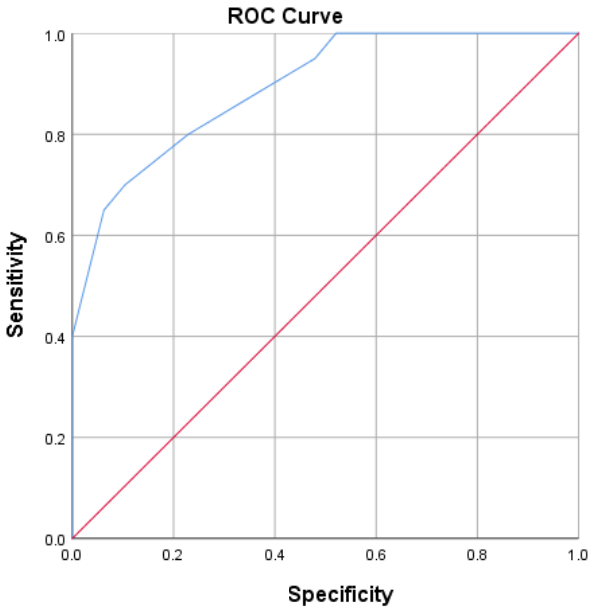


Fig. 3.3. ROC curve for *C. siamensis* response rate, with caller body size and sex as predictor variables.

3.2.4 Binomial logistic regression (BLR) for *O. tetraspis* (Bristol)

The BLR model for the *O. tetraspis*, Bristol population was statistically significant ($\chi2(2) = 8.381$, $P = 0.015$; Table 3.2). Caller body size was a significant predictor variable, with the 60cm juvenile distress call eliciting the lowest response rate (see ' Influence of Distress Callers' Body Size'). There were two standardized residuals above the value of three standard deviations which were investigated and kept. The area under the ROC curve was 0.738 (95% C.I. 0.582 - 0.894) which is an acceptable level of discrimination according to Hosmer *et al.* (2013) (Fig. 3.4).

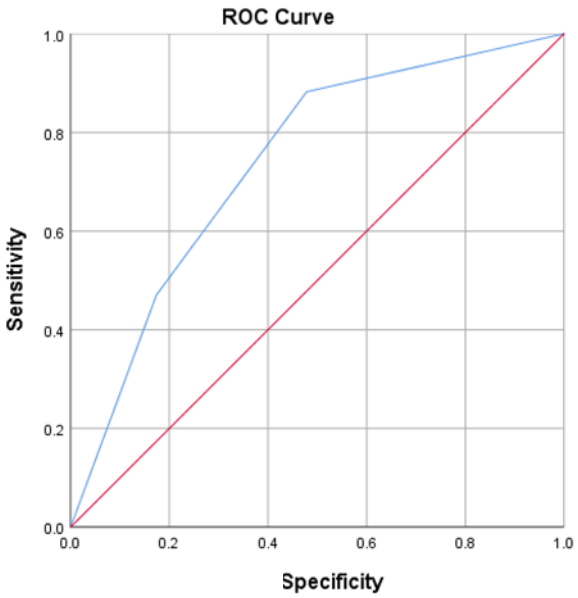


Fig. 3.4. ROC curve for *O. tetraspis* (Bristol population) response rate, with caller body size as a predictor variable.

3.2.5 Binomial logistic regression (BLR) for *O. tetraspis* (Oxford)

The BLR model for the *O. tetraspis*, Oxford population was statistically significant; $\chi 2(2) = 17.24$, $P < 0.001$ (Table 3.2). Two predictor variables were statistically significant, sex (see 'Influence of Sex on Level of Care') and breeding period (see 'Influence of Breeding Period'). The female was more likely to respond to a distress call over the male. Additionally, during the breeding period there was a higher response rate from both sexes compared to the non-breeding period. There was one standardized residual above the value of 3 standard deviations which was kept in the data. The area under the ROC curve was 0.888 (95% C.I. 0.726 - 0.941), an excellent level of discrimination according to Hosmer *et al.* (2013)(Fig. 3.5).

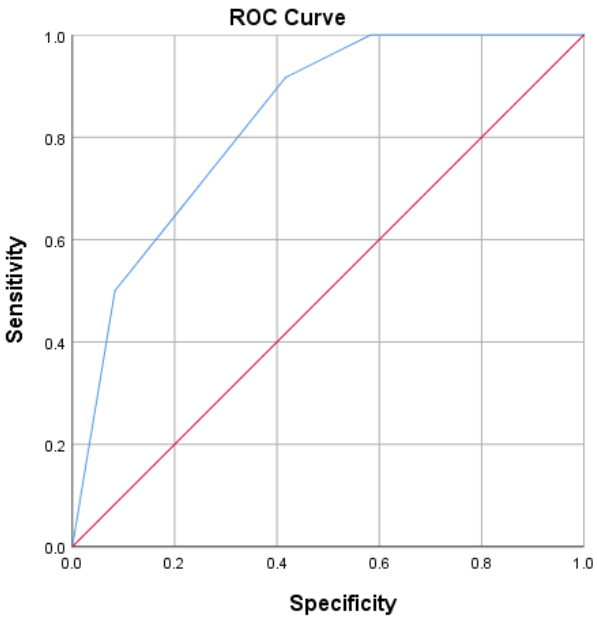


Fig. 3.5. ROC curve for *O. tetraspis* (Oxford population) response rate, with breeding period and sex as predictor variables.

3.3 Strength of Response

The most common type of response elicited from a distress call was 0 (no response; N = 269; Fig. 3.6).The least common response type was -1 (evasive response; N = 5). Evasive responses (-1) only occurred in the *C. niloticus*, *C. rhombifer* and *T. schlegelii* populations in response to a 160 cm or 60 cm juvenile caller (Table 3.3).

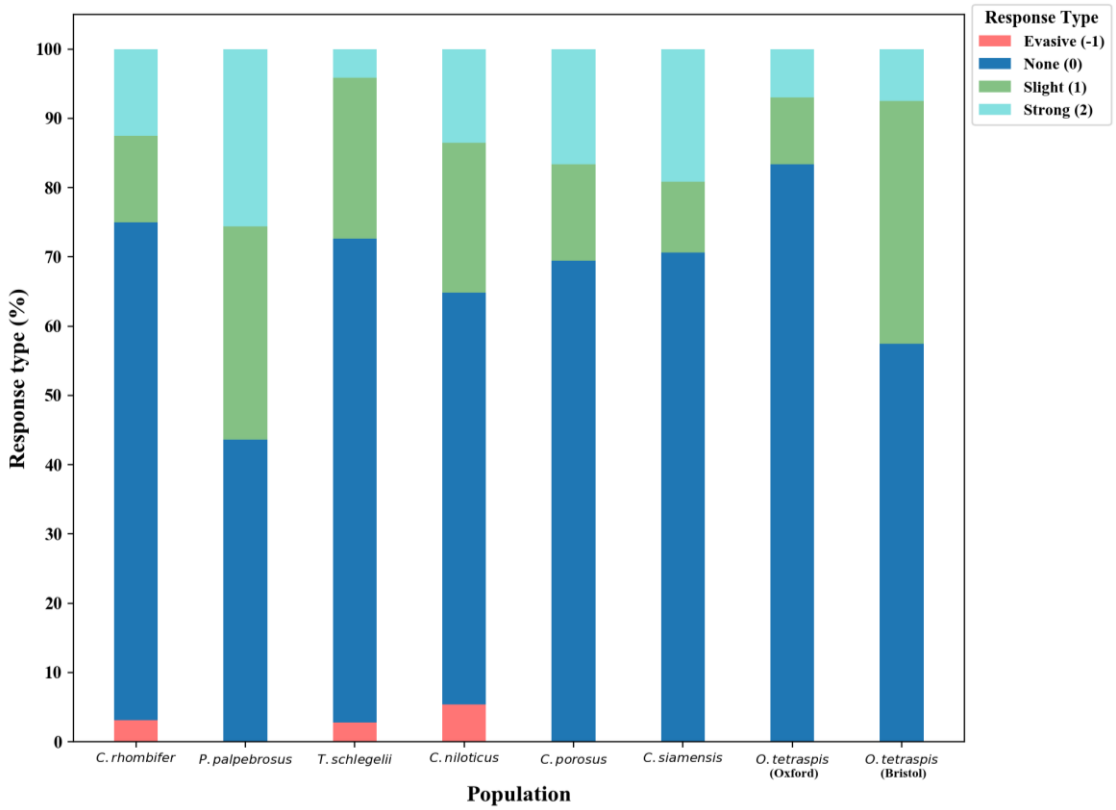


Fig. 3.6. Total percentage occurrence of each response type for all sample populations.

Table 3.3. Number of occurrences of each type of response strength elicited from a distress call for all sampled population. Note: -1 = evasive response, 0 = no observable response, 1 = slight response, 2 = strong response.

Population	Response Type				Total
	-1	0	1	2	
C. rhombifer	1	23	4	4	32
P. palpebrosus	0	17	12	10	39
T. schlegelii	2	51	17	3	73
C. niloticus	2	22	8	5	37
C. porosus	0	25	5	6	36
C. siamensis	0	48	7	13	68
O. tetraspis (Oxford)	0	60	7	5	72
O. tetraspis (Bristol)	0	23	14	3	40
Total	5	269	74	49	397

3.4 Influence of Breeding Period

3.4.1 Effect of breeding period on response rate

The binomial logistic regression (BLR) and generalized estimating equation (GEE) were run on the response rate data for actively breeding populations. Sex, caller body size and species were predictor variables. According to these models, the effect of breeding period on response rate was not significant when the populations were sampled together. However, there were significant differences in response rates between the species (GEE: N = 237, Wald $\chi2(3) = 111.89$, $P < 0.001$). Additionally, the response rates between the two *O. tetraspis* populations were statistically significantly different (GEE: N= 112, Wald $\chi2(3) = 6$, $P = 0.028$). Therefore, further regressions were run on each population independently. To summarise, the GEE results reported that breeding period had a significant effect on response rate for the *T. schlegelii* population, with response rate increasing during the breeding period. The results also found a significant decrease in response rate during the breeding period for the *O. tetraspis* populations. The results from the BLR analysis concurred that breeding period had a significant effect on response rate for the *T. schlegelii* and *O. tetraspis* Bristol population, but not in the Oxford population. There were no significant differences in response rate between the breeding and non-breeding period for the *C. siamensis* population.

3.4.2 Effect of breeding period on response rate of *T. schlegelii*

The results of the GEE and BLR found that response rate was significantly higher during the breeding period compared to the non-breeding period for the *T. schlegelii* population (N = 72, GEE: Wald $\chi2(1) = 432.96$, $P < 0.001$ & BLR: $\chi2(2) = 36.89$, $P < 0.001$). The BLR analysis reported that odds of response were 20.3 times higher during the breeding period (Table 3.4.). Additionally, the GEE analysis found that the estimated mean response rate was significantly higher during the breeding period (EM=0.39, Wald 95% C.I = 0.35 – 0.43) compared to the non-breeding period (EM=0.04, Wald 95% C.I = 0.04 - 0.05; Table 3.5., Fig. 3.7).

Table 3.4. BLR output for *T. schlegelii* and *O. tetraspis* (Oxford) response rate during the breeding and non-breeding period. Note: S.E.= standard error. B= unstandardized regression coefficient (Non-breeding period is the intercept).

						50% Confidence Intervals for Odds Ratio	
Population	**B**	**S.E.**	**Wald**	***p***	**Odds Ratio**	**Lower**	**Upper**
T. schlegelii	3.00	0.88	11.59	0.001	20.30	11.18	36.86
O. tetraspis (Oxford)	-1.75	0.75	5.47	0.019	0.17	0.11	0.29

Table 3.5. GEE (binomial model) output for response rate during the breeding and non-breeding period. Note: S.E.= standard error. B= unstandardized regression coefficient (breeding period is the intercept).

Population	**B**	**S.E.**	**95% Wald C.I.**		**Wald Chi Square**	***P***	**Estimated Mean for Breeding Period**	**Estimated Mean for Non-breeding Period**
			Lower	**Upper**				
T. schlegelii	-2.75	0.13	-3.01	-2.49	432.96	0.001	0.39	0.04
O. tetraspis (Bristol)	2.56	1.03	0.55	4.57	6.23	0.013	0.08	0.53
O. tetraspis (Oxford)	0.67	0.015	0.65	0.70	2138.49	0.001	0.05	0.10

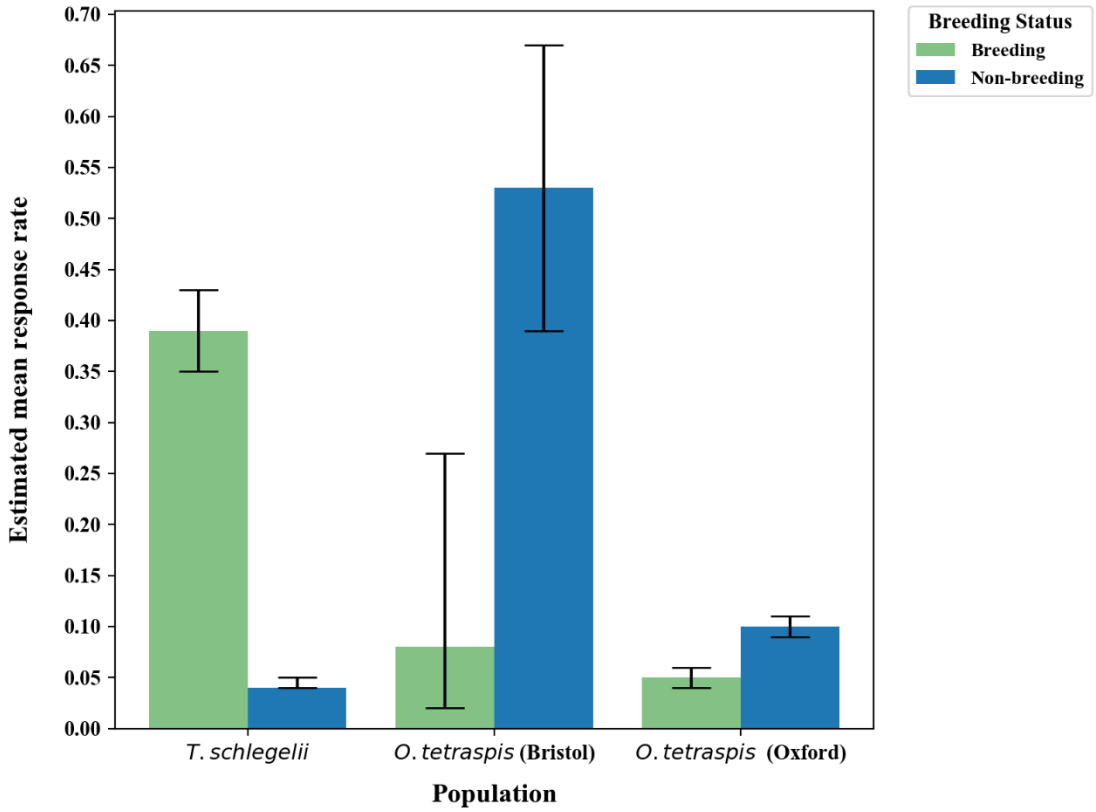


Fig. 3.7. Estimated mean ± SD response rates for *T. schlegelii* and *O. tetraspis* for the breeding and non-breeding period.

3.4.3 Effect of breeding period on response rate of *O. tetraspis* (Bristol population)

The Bristol population of *O. tetraspis'* response rate was significantly lower during the breeding period compared to the non-breeding period according to the GEE analysis (N = 40, Wald $\chi2(1) = 6.225$, $P = 0.013$). Estimated mean response rate was 0.08 (Wald 95% C.I = 0.02 - 0.27) during the breeding period and 0.53 (Wald 95% C.I = 0.39 - 0.67) for the non-breeding period (Table 3.5., Fig. 3.7). However, the variable that accounts for habituation (number of playback sessions) was also significant, with response rate decreasing as playback exposure increased (GEE: Wald $\chi2(1) = 8.459$, $P = 0.004$). Contrastingly, the BLR model reported no significant difference in response rate between the breeding and non-breeding period for the Bristol population.

3.4.4 Effect of breeding period on response rate of *O. tetraspis* (Oxford population)

The GEE and BLR results for the Oxford population of *O. tetraspis* reported that response rate was significantly lower during the breeding period (N=71, GEE: Wald $\chi2(1) = 2138.45$, $P = 0.001$, & BLR: $\chi2(2) = 8.38$, $P = 0.019$). The estimated mean response rate was 0.05 (Wald 95% C.I = 0.04 – 0.06) during the breeding period and 0.10 (Wald 95% C.I = 0.09 – 0.11) for the non-breeding period (Fig. 3.7). The BLR predicted 0.17 times lower odds of response during the breeding period compared to the non-breeding period (Table 3.4.). According, to the GEE analysis, the number of playback sessions was significant as response rate decreased as playback exposure increased (Wald $\chi2(1) = 16.11$, $P < 0.001$).

3.4.5 Effect of breeding period on reaction time

A multi-linear regression model (MLR) and GEE (linear model) were run on the reaction time data and concluded that reactions to distress calls for all actively breeding populations were significantly faster during the breeding period (N = 71, MLR: $F(2,68) = 13.81$, $P < 0.001$, & GEE: Wald $\chi2(1) = 6.26$, $P = 0.012$). The MLR predicted reaction time to be 5.83 s faster during the breeding period (Table 3.1). The GEE estimated mean reaction time for the breeding period was 7.52 s (Wald 95% C.I = 7.00 – 8.20) and 11.91 s (Wald 95% C.I = 11.00 – 12.30) for the non-breeding period (Table 3.6). Individual tests for each population were not run due to lack of positive responses (N = 71).

Table 3.6. GEE (linear model) output for reaction time with sex and breeding period as predictor variables. Note: B= unstandardized regression coefficient. S.E.= standard error. Breeding period and male are the intercept.

Variable	B	S.E.	95% Wald Confidence Interval		Wald Chi-Square	*p*	Estimated Mean	95% C.I for Estimated Mean	
			Lower	Upper				Lower	Upper
Non-breeding Period	4.38	1.75	0.95	7.82	6.26	0.012	11.91	8.05	15.35
Breeding Period	0[a]	.	.	.	.	.	7.52	4.20	10.65
Female	-8.02	2.95	-13.80	-2.23	7.38	0.007	7.50	5.55	11.15
Male	0[a]	.	.	.	.	.	18.98	14.05	22.95

3.5 Influence of Sex on Level of Care

3.5.1 Effect of sex on response rate

There was a significant difference in response rate between the sexes among the four populations tested (GEE: N= 237, Wald $\chi 2(1) = 25.20$, $P < 0.001$). The females were predicted to have a 7.40 higher chance of responding to distress calls compared to males (Table 3.7, Fig. 3.8). There was a significant difference between the populations so further regression models were run on each population independently. To summarise, the GEE analysis found significant differences in response rate between the sexes for all populations where sex could be determined (Table 3.8). The BLR analysis found significant differences in response rate between the sexes in all but the Bristol population of *O. tetraspis* (Table 3.7).

Table 3.7. BLR output for likelihood of response to a distress call with sex as a predictor variable. Note: B= unstandardized regression coefficient. S.E.= standard error. Male is the intercept.

Population	B	S.E.	Wald	*p*	Odds Ratio	50% C.I. for Odds Ratio	
						Lower	Upper
All	2.00	0.40	25.20	0.001	7.40	3.39	16.18
T. schlegelii	3.49	0.87	16.12	0.001	32.75	18.22	58.85
C. siamensis	2.02	1.12	3.23	0.072	7.50	3.52	15.98
O. tetraspis (Oxford)	2.97	1.12	7.06	0.008	19.50	9.17	41.43

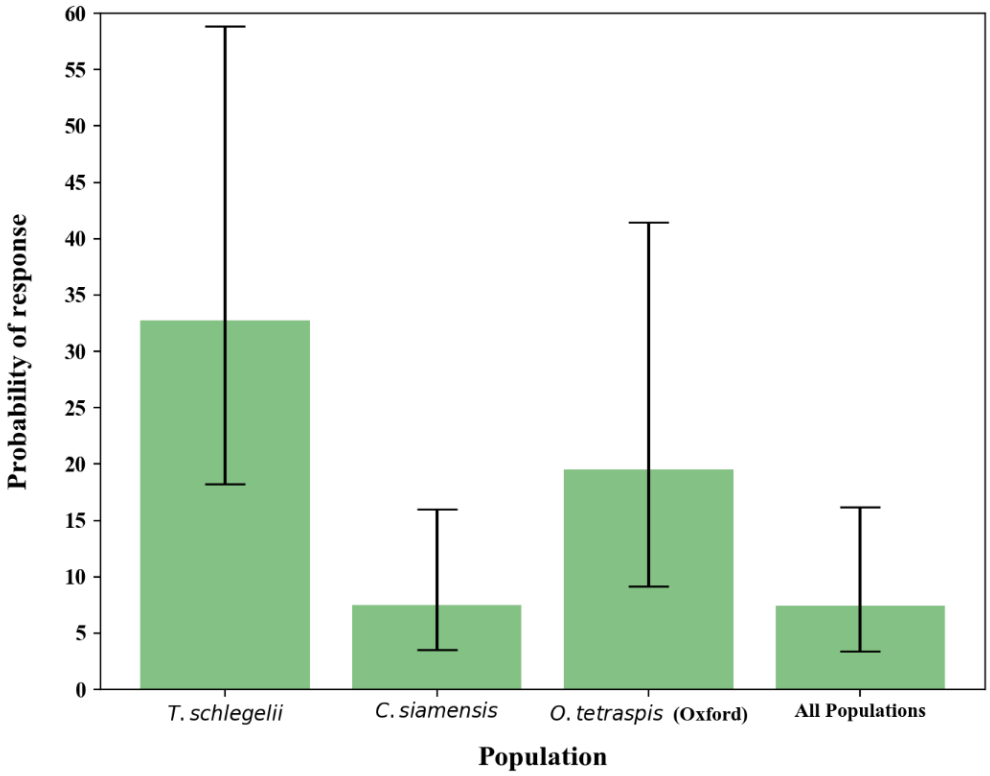


Fig. 3.8. Probability of females responding to distress calls over males for *T. schlegelii, C. siamensis* and *O. tetraspis* (50% ± SD).

Table 3.8. GEE (binomial model) output for response rate to distress calls with sex as a predictor variable. Note: B= unstandardized regression coefficient. S.E.= standard error. Males are the intercept.

Population	B	S.E.	95% Wald Confidence Interval		Wald Chi-Square	*p*	Estimated Mean for Females	Estimated Mean for Males
			Lower	Upper				
All	2.00	0.29	1.43	2.58	46.80	<0.001	0.39	0.08
T. schlegelii	3.27	0.02	3.12	3.21	19293.30	<0.001	0.36	0.02
C. siamensis	2.54	0.30	1.95	3.13	70.93	<0.001	0.47	0.06
O. tetraspis (Bristol)	1.48	0.10	1.28	1.69	196.79	<0.001	0.40	0.13
O. tetraspis (Oxford)	3.26	0.17	2.90	3.58	356.97	<0.001	0.29	0.02

3.5.2 Effect of sex on response rate of *T. schlegelii*

The *T. schlegelii* population had the largest difference in response rate between the sexes out of the four populations tested (N = 72, GEE: Wald $\chi2(1) = 19293.30$, $P < 0.001$ & BLR: $\chi2(2) = 36.89$, $P < 0.001$). The female was predicted to have 32.75 times higher odds of exhibiting a response to a distress call compared to the male (Table 3.7, Fig. 3.8). This was supported by the results from the GEE as estimated mean response was significantly higher for the female compared to the male (Table 3.8).

3.5.3 Effect of sex on response rate of *C. siamensis*

The female *C. siamensis* had 12.64 times higher odds of responding to a distress call compared to the male (N=51, $\chi2(5) = 35.99$, $P = 0.021$; Fig. 3.8). The estimated mean response rate for the female was also significantly higher than the male (N=51, Wald $\chi2(2)$ 70.93, $P < 0.00$; Table 3.8).

3.5.4 Effect of sex on response rate of *O. tetraspis* (Bristol population)

In the Bristol population of *O. tetraspis,* The GEE estimated the mean response rate to be significantly higher for the female compared to the male (N = 40, Wald $\chi2(1) = 196.79$, $P < 0.001$; Table 3.8).

3.5.5 Effect of sex on response rate of *O. tetraspis* (Oxford population)

For the Oxford population of *O. tetraspis* the female was predicted to have 19.50 times higher odds of response to a distress call compared to the male (N= 71, BLR: $\chi2(2) = 17.238$; $P = 0.008$; Table 3.7 & GEE: Wald $\chi2(1) = 356.97$, $P < 0.001$; Table 3.8, Fig. 3.8).

3.5.6 Effect of sex on reaction time

The MLR analysis reported that reaction time was significantly influenced by sex (N = 71, F (2,68) = 13.81, $P < 0.001$) and predicted a 5.83 s faster reaction time for females compared to males (Table 3.1). The GEE (linear model) also reported that sex was statistically significant when determining reaction time to distress calls ($\chi2(1) = 7.378$, $P = 0.007$). The estimated mean reaction time to a distress call was calculated as 7.50 s (Wald 95% C.I =5.45- 9.75) for females and 18.98 s (Wald 95% C.I = 13.24 - 24.02) for males (Table 3.6).

3.6 Influence of Distress Callers' Body Size

3.6.1 Effect of caller body size on response rate

When grouping all populations together neither the BLR or the GEE analysis found significance when comparing response rates to the 20 cm, 60 cm and 160 cm sized distress callers. Individual BLR tests were run on each population, according to the BLR results, only the Bristol population of *O. tetraspis* had significant differences in response rates between caller body size (N= 40, $\chi 2$ (2) = 6.595, P= 0.037, Table 3.9). Contrastingly, according to the GEE results there was significance between caller body size in both populations of *O. tetraspis* (Bristol: N = 40,Wald $\chi 2$ (1) = 101.38, P= 0.023, Table 3.10 & Oxford: N=71, Wald $\chi 2$ (1) =16.05, P= 0.006, Fig. 3.9) and *C. rhombifer* (N=32, Wald $\chi 2$ (1) = 7.54, P = 0.006, Fig. 3.9). There was no significant difference found in response rate to caller body size in the *P. palpebrosus*, *C. porosus*, *C. niloticus* and *C. siamensis* population.

Table 3.9. BLR output for response rate of *O. tetraspis* (Bristol population) when the predictor variable is caller body size. Note: B= unstandardized regression coefficient. S.E.= standard error. 60 cm is the intercept.

Caller body size	B	S.E.	Wald	*p*	Odds Ratio	95% C.I. for Odds Ratio	
						Lower	Upper
All	-	-	6.60	0.037	-	-	-
20 cm	2.49	0.98	6.44	0.011	12.00	1.76	81.75
160 cm	1.79	0.93	3.69	0.055	6.00	0.97	37.30

Table 3.10. GEE output for response rate of *O. tetraspis* (Bristol population) when the predictor variable is caller body size. Note: B= unstandardized regression coefficient. S.E.= standard error. 60 cm is the intercept.

Caller body size	B	S.E.	95% Wald C.I.		Wald Chi-Square	*p*	Estimated Mean	95% Wald C.I. For Estimated Mean	
			Lower	Higher				Lower	Upper
20 cm	3.30	0.33	2.66	3.93	101.38	<0.001	0.64	0.64	0.65
160 cm	2.38	0.30	1.80	2.96	63.91	<0.001	0.42	0.17	0.72
60 cm	0[a]	-	-	-	-	-	0.06	0.03	0.12

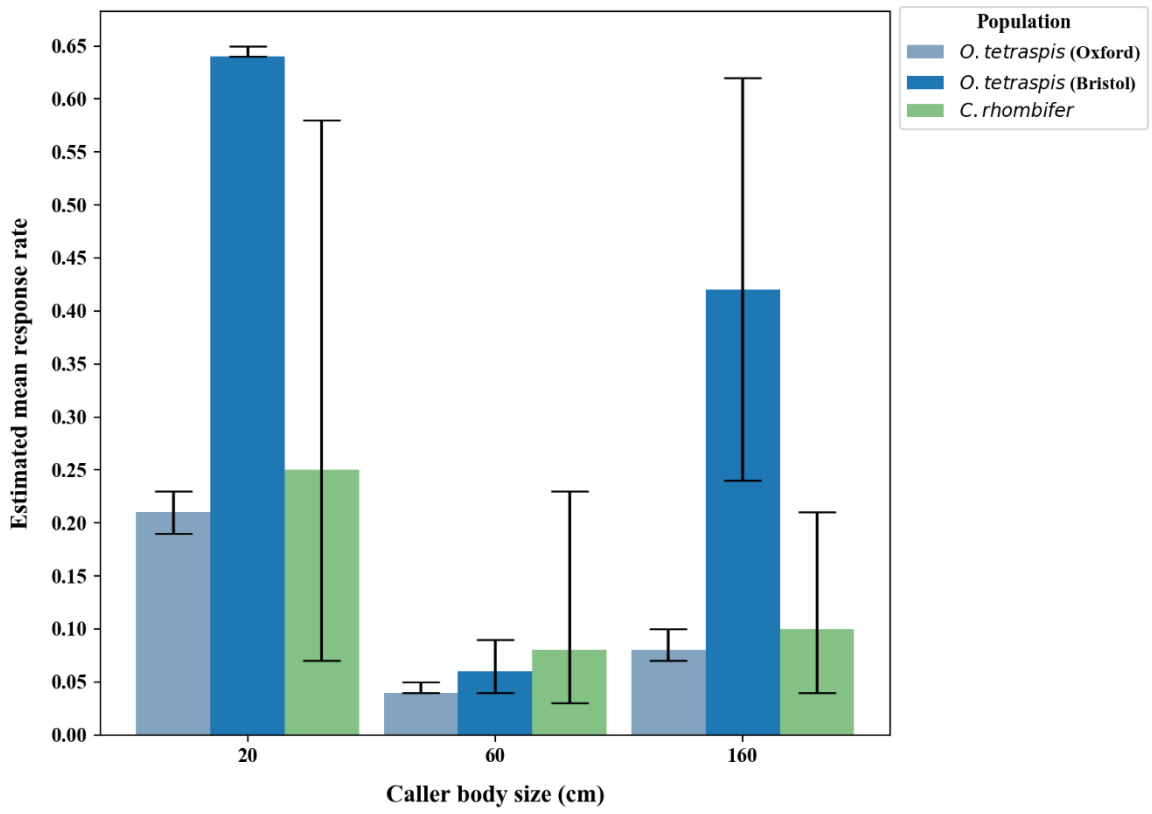


Fig. 3.9. Estimated mean (95% ± SD) response rate of *C. rhombifer* and *O.tetraspis* to 20 cm, 60 cm and 160 cm sized juvenile distress callers.

3.6.2 Effect of caller body size on response rate of *O. tetraspis* (Bristol population)

The odds of response were predicted to be 12 times higher for the 20 cm sized caller and 6 times higher for the 160 cm sized compared to the 60 cm sized caller in the Bristol population of *O. tetraspis* (Table 3.9). According to the GEE analysis, estimated mean response rate was highest when individuals were played a distress call from a 20 cm sized caller (EM = 0.64, Wald 95% C.I = 0.64 - 0.65), and lowest for a 60 cm sized caller (EM= 0.06, Wald 95% C.I =0.03- 0.12; Table 3.10 & Fig. 3.9). The estimated mean response rate for the 160 cm distress caller was 0.42 (Wald 95% C.I = 0.17- 0.72; Fig. 3.9).

3.6.3 Effect of caller body size on response rate of *O. tetraspis* (Oxford Population)

The GEE analysis found a significant difference in response rate to the size of juvenile distress callers in the Oxford population of *O. tetraspis* (Table 3.11, Fig. 3.9). According to the GEE results, estimated mean response rate was highest for the 20 cm juvenile (EM = 0.21, Wald 95% C.I = 0.18 - 0.24) and lowest for the 60 cm juvenile (EM = 0.04, Wald 95% C.I = 0.03 - 0.06; Fig. 3.9). However, the results of the BLR analysis reported no significant differences in response rates.

Table 3.11. GEE output for *O. tetraspis* (Oxford population) response rate with caller body size (cm) as the predictor variable. Note: B= unstandardized regression coefficient. S.E.= standard error. 20 cm sized caller is the intercept.

Caller body size	B	S.E.	95% Wald C.I.		Wald Chi-Square	*p*	Estimated Mean Response	95% Wald C.I For Estimated Mean	
			Lower	Higher				Lower	Upper
160 cm	-1.08	0.27	-1.60	-.550	16.05	<0.001	0.08	0.06	0.11
60 cm	-1.77	0.24	-2.24	-1.29	53.27	<0.001	0.04	0.03	0.06
20 cm	0[a]	-	-	-	-	-	0.21	0.18	0.24

3.6.4 Effect of caller body size on response rate of *C. rhombifer*

There was a significant difference in response rate to the size of juvenile distress callers in the *C. rhombifer* population according to the GEE analysis (N= 32, Table 3.12, Fig. 3.9). Estimated mean response rate was highest for the 20 cm juvenile (EM = 0.25, Wald 95% C.I = 0.03 - 0.75) and lowest for the 60 cm juvenile (EM = 0.08, Wald 95% C.I = 0.02 - 0.35; Table 3.12). Playback session number was also significant (Wald $\chi 2$ (1) = 4.35, P= 0.037). Contrastingly, there was no significant difference in response rate reported by the BLR analysis.

Table 3.12. GEE output for *C. rhombifer* response rate with caller body size (cm) as a predictor variable. Note: B= unstandardized regression coefficient. S.E.= standard error. 20 cm sized caller is the intercept.

Caller body size	B	S.E.	95% Wald C.I.		Wald Chi-Square	*p*	Estimated Mean Response	95 % Wald C.I. of Estimated Mean	
			Lower	Higher				Lower	Upper
160 cm	-1.13	0.41	-1.93	-0.32	7.54	0.006	0.10	0.03	0.31
60 cm	-1.29	0.22	-1.72	-0.85	33.44	<0.001	0.08	0.02	0.35
20 cm	0[a]	-	-	-	-	-	0.25	0.03	0.75

3.6.5 Effect of caller body size on reaction time

When analysing all populations together neither the GEE (linear model) or the MLR analysis found significance between caller body size and reaction time. As number of positives responses were lacking (N = 127), analyses could not be run on an individual populations' reaction time. However, there was a significant difference in reaction time for the non-breeding populations between the 20 cm and 160 cm sized callers (Table 3.13).

Table 3.13. GEE (linear model) output for reaction times of non-active breeders between 20 cm and 160 cm distress callers. Note: B= unstandardized regression coefficient. S.E.= standard error. 160 cm is the intercept.

Juvenile Size	B	S.E.	95% Wald Confidence Interval		Wald Chi-Square	*df*	*p*	Estimated Mean Reaction Time (s)
			Lower	Upper				
20 cm	-2.25	0.73	-3.68	-0.82	9.46	1	0.002	6.50
160 cm	0[a]	.	.	.	.	.	.	8.75

3.7 Kin Discrimination

3.7.1 Effect of kin on response rate of *C. siamensis*

The *C. siamensis* population had a significantly higher response rate to their offspring's distress calls compared to the *C. niloticus* distress calls (N = 68; BLR: $\chi 2$ (5) = 12, P= 0.007 & GEE: Wald $\chi 2$ (1) = 9.984, P= 0.002). According to the BLR analysis, the 20 cm *C. niloticus* caller had 39 times lower odds of eliciting a response than the *C. siamensis'* offspring caller (Table 3.14, Fig. 3.10). Additionally, a 60 cm and 160 cm *C. niloticus* call had 34.7 times and 15.9 times lower odds of receiving a response respectively (Table 3.14). The estimated mean response rate was highest for offspring calls and lowest for the 20 cm *C. niloticus* distress caller (Table 3.15).

Table 3.14. BLR output for *C. siamensis'* response rate to *C. niloticus* distress calls (20 cm, 60 cm and 160 cm) compared to offspring distress calls (55 cm). Note: B= unstandardized regression coefficient. S.E.= standard error. Offspring is the intercept.

Caller body size	B	S.E.	Wald	*P*	Odds Ratio	50% C.I. for Odds Ratio	
						Lower	Upper
All	-	-	12.00	0.007	-	-	-
20 cm	3.66	0.98	13.90	<0.001	39	20.1	75.67
60 cm	3.55	0.99	12.93	<0.001	34.67	17.82	67.43
160 cm	2.77	0.91	9.17	0.002	15.89	8.58	29.42

Table 3.15. GEE (binomial model) output for *C. siamensis* response rate to unrelated young distress calls (20 cm, 60 cm and 160 cm) compared to offspring distress calls (55 cm). Note: B= unstandardized regression coefficient. S.E.= standard error. Offspring is the intercept.

Caller body size	B	S.E.	95% Wald C.I.		Wald Chi-Square	*p*	Estimated Mean Response	95% Wald C.I. For Estimated Mean	
			Lower	Upper				Lower	Upper
20 cm	-4.71	1.49	-7.62	-1.79	9.98	0.002	0.05	0.01	0.18
60 cm	-4.54	1.45	-7.38	-1.69	9.78	0.002	0.06	0.02	0.19
160 cm	-3.64	0.88	-5.36	-1.92	17.14	<0.001	0.14	0.12	0.16
Offspring	0[a]	-	-	-	-	-	0.86	0.57	0.97

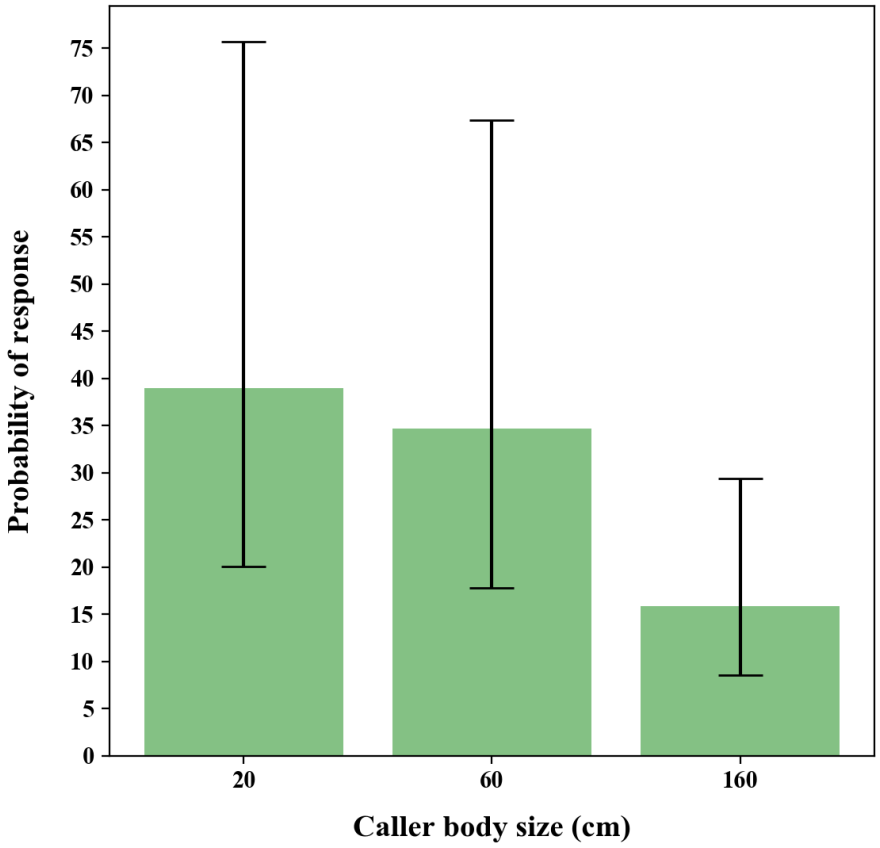


Fig. 3.10. Probability of *C. siamensis* responding to their offspring's distress call (55 cm) over a 20 cm, 60 cm and 160 cm *C. niloticus's* distress call (50% ± SD).

3.7.2 Effect of kin on response rate of *P. palpebrosus*

According to the GEE analysis there was no significant difference in *P. palpebrosus* response rate between their offspring (45 cm) and the non-related juvenile calls when non-related calls were grouped into one variable (N= 39, Wald $\chi 2$ (1) = 2.119, P= 0.138). However, there was a significantly different response rate to the 60 cm body sized callers (P= 0.010) and the 160 cm sized callers (P< 0.001) in comparison to their offspring's distress calls (Table 3.16). Estimated mean response rate was highest for offspring distress callers (E.M = 0.88) and lowest for the 160 cm non-related distress callers (E.M = 0.31). The BLR analysis also reported a significant difference in response rate between the distress calls of offspring and non-related juveniles (N=39, $\chi 2$ (3) = 8.308, P= 0.045). Predicted odds of response were 15.8 times higher when the sample were played their offspring's distress calls (Table 3.17).

Table 3.16. GEE output for *P. palpebrosus* response rate between unrelated young and offspring distress calls (45 cm). Note: B= unstandardized regression coefficient. S.E.= standard error. Offspring is the intercept.

Caller body size	B	S.E.	95% Wald C.I.		Wald Chi-Square	*p*	Estimated Mean Response	95% Wald C.I For Mean Response	
			Lower	Upper				Lower	Upper
20 cm	-	-	-	-	-	0.138	0.70	0.55	0.82
60 cm	-1.95	0.76	-3.43	-0.46	6.60	0.010	0.50	0.33	0.67
160 cm	-2.76	0.19	-3.12	-2.39	221.60	<0.001	0.31	0.12	0.59
Offspring							0.88	0.76	0.94

Table 3.17. BLR output for *P. palpebrosus* response rate to 160 cm unrelated young distress calls compared to their offspring's distress calls (45 cm). Note: B= unstandardized regression coefficient. S.E.= standard error. 160 cm unrelated distress caller is the intercept.

Caller body size	B	S.E.	Wald	*p*	Odds Ratio	50% C.I. for Odds Ratio	
						Lower	Upper
Offspring	2.76	1.23	5.05	0.025	15.75	6.89	36.02

3.8 Relative Difference in Body Size Between Caller and Receiver

A Mann-Whitney U test and GEE (ordinal logistic model) were run to determine if there were significant differences in response strength between relatively large and small receivers (N=373, Fig. 3.11). The Mann-Whitney U test and GEE reported significant differences in response strength when populations were played a distress call belonging to a 20 cm juvenile but not for 60 cm and 160 cm sized juveniles. The Mann-Whitney U test reported that response strength was significantly higher (N= 131, U = 2.63, z = 2.83, P= 0.005) for the relatively small body size group (mean rank = 74.59) than the

large body size group when comparing responses to 20cm distress callers (mean rank = 58.96; Fig. 3.12). However, distributions of response strength between the two groups were dissimilar, as assessed by visual inspection. Therefore, results should be regarded with caution. However, the GEE results supported the Mann-Whitney U test as it reported a larger estimated mean response to the 20 cm long distress callers from the small bodied group (N=131, Wald $\chi 2$ (1) = 7.18, P= 0.007; Table 3.18).

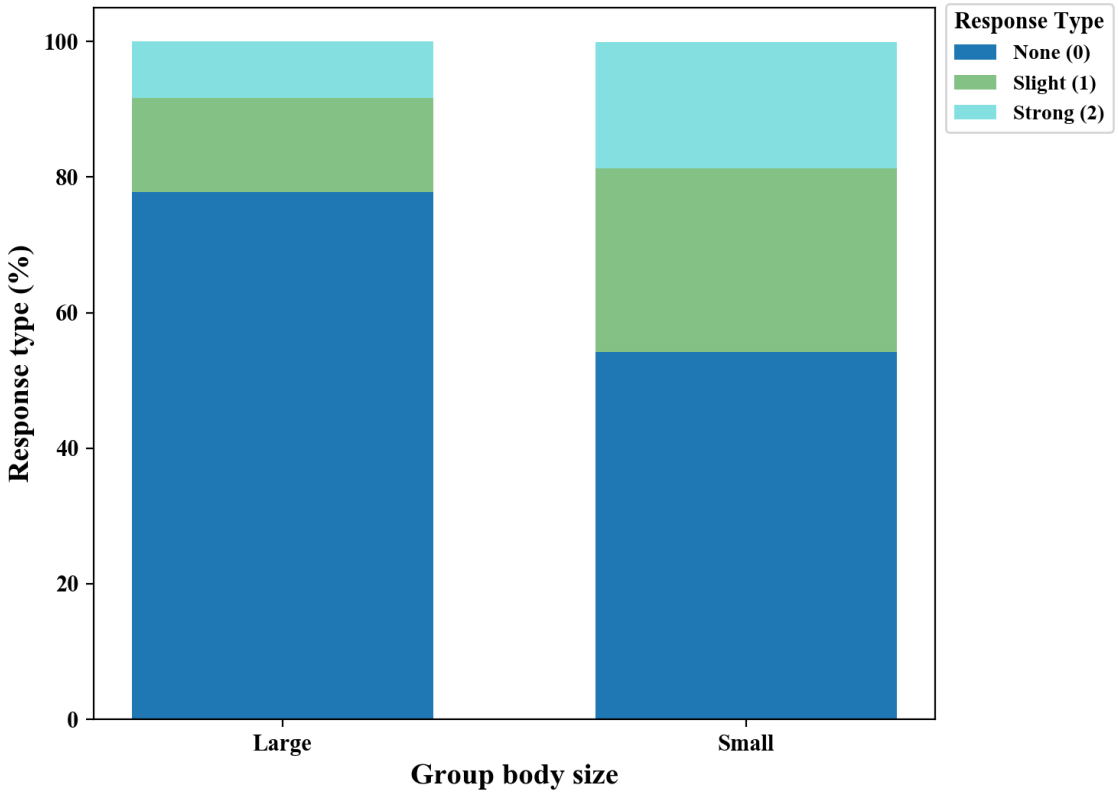


Fig. 3.11. Total percentage (%) of each recorded response type elicited from small-body size and large-body size populations.

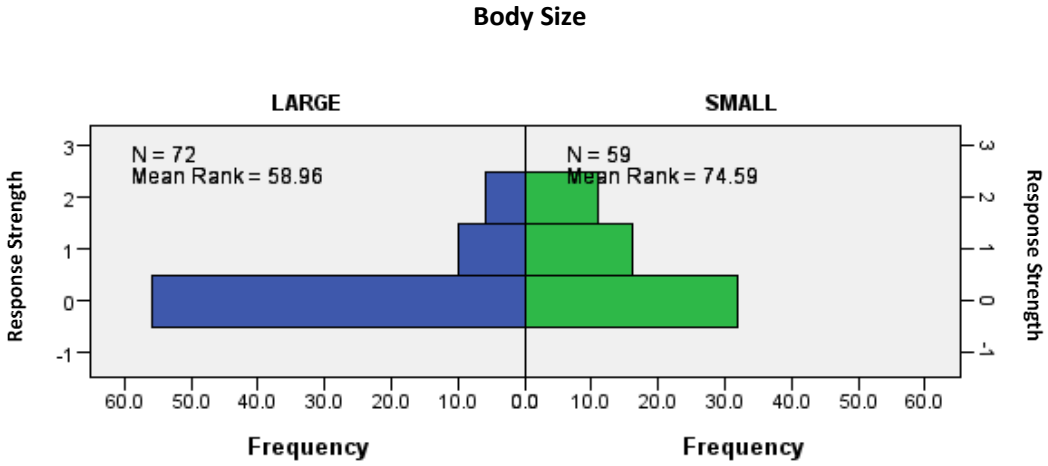


Fig. 3.12. Response strength to 20 cm distress callers between small-body size and large-body size groups.

Table 3.18. GEE (ordinal model) output for response strength to 20 cm distress callers between small and large receivers. Note: B= unstandardized regression coefficient. S.E.= standard error. Small body size is the intercept.

Body Size	B	S.E.	95% Wald Confidence Interval		Wald Chi-Square	*p*
			Lower	Upper		
Large	-1.02	0.38	-1.77	-0.27	7.18	0.007
Small	0[a]	.	.	.	.	.

3.9 Influence of Breeding Sociality

The Mann-Whitney U test found significant differences in response strength between social and non-social breeding strategists (Fig. 3.13; N = 289, U = 10,810, z = 2.128, P= 0.033). Response strength was statistically significantly higher in social breeders (mean rank=156.45) than non-social breeders (mean rank=138.56). Distribution of response strength for these two groups were similar, as assessed by visual inspection (Fig. 3.13). The GEE analysis also found a significant difference between response strength and breeding strategies, with social breeders having a stronger response to distress calls (N= 289, Wald $\chi 2$ (1) = 4.511, P= 0.034; Fig. 3.14; Table 3.19).

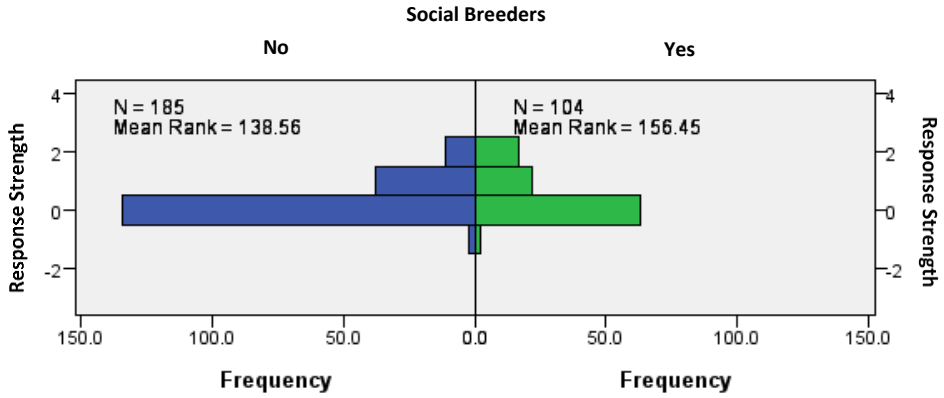


Fig. 3.13. Response strength to distress calls between social and non-social breeders.

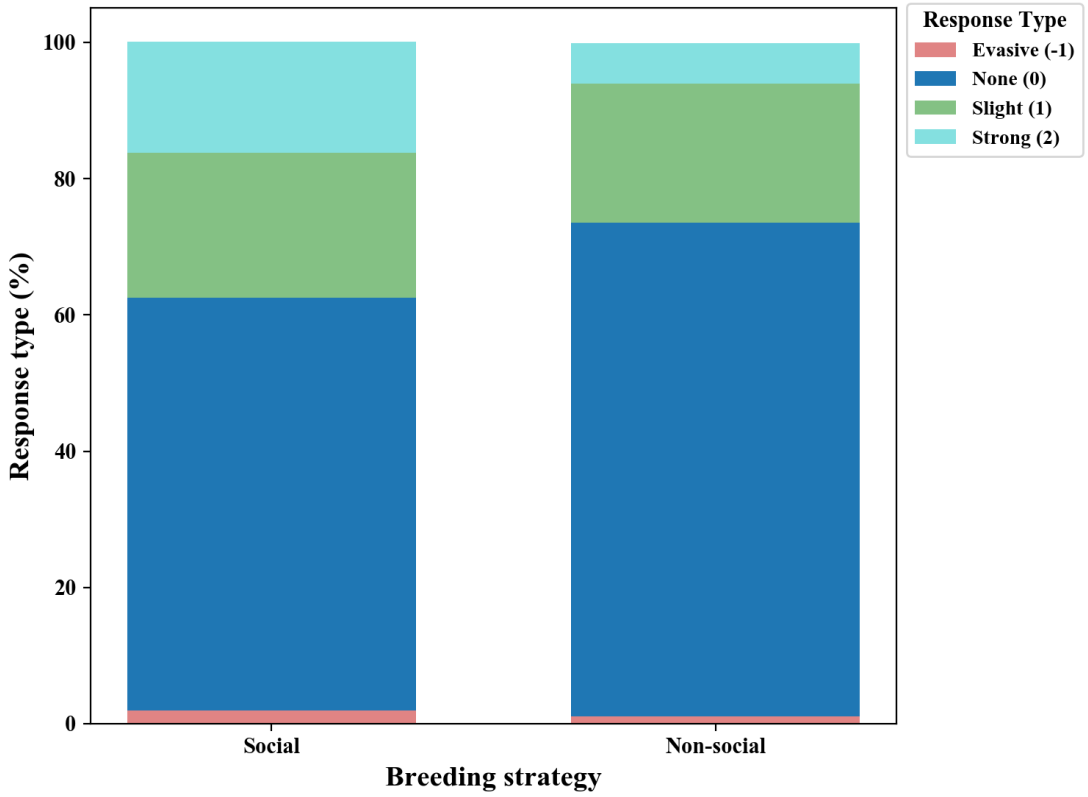


Fig. 3.13. Total percentage (%) of each recorded response type elicited from social and non-social breeders.

Table 3.19. GEE (ordinal model) output of response strength to distress calls (20 -160 cm) between social and non-social breeding strategies. Note: B= unstandardized regression coefficient. S.E.= standard error. Social breeding strategy is the intercept.

Breeding strategy	B	S.E.	95% Wald Confidence Interval		Wald Chi-Square	*p*
			Lower	Upper		
Non-social breeders	-0.59	0.28	-1.13	-0.05	4.51	0.034

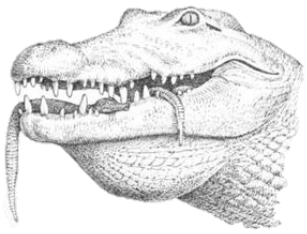
3.10 Pairwise Comparison of Species' Response Rates

The GEE was run on the response rate data and the analysis found significant differences between all populations (Table 3.20). For comparison, only the responses from the 20 cm, 60 cm and 160 cm sized juveniles were used in this model. The *C. siamensis, O. tetraspis* (Oxford population) and *C. rhombifer* response rates were significantly different from all the sampled populations.

Table 3.20. Significance matrix generated by GEE for all sample populations using response rate data. Note: only the 20, 60 and 160 cm sized juvenile calls were used in this model. Population, size of caller and playback session number were the predictor variables. Statistically significant differences in responses are highlighted in green. N.A = not significant.

Population	*C. rhombifer*	*P. palpebrosus*	*T. schlegelii*	*C. niloticus*	*C. porosus*	*C. siamensis*	*O. tetraspis* (Oxford)
O. tetraspis (Bristol)	<0.001	N.A	0.019	N.A	N.A	<0.001	<0.001
C. rhombifer		<0.001	<0.001	<0.001	0.004	<0.001	<0.001
P. palpebrosus			<0.001	<0.001	<0.001	<0.001	<0.001
T. schlegelii				<0.001	N.A	<0.001	<0.001
C. niloticus					<0.001	<0.001	<0.001
C. porosus						<0.001	<0.001
C. siamensis							0.031

4. Discussion



This is the first study into the effects of breeding period, socialness of breeding strategy, relatedness to the caller, sex, species and relative size on crocodilian responses to juvenile distress calls. In this study, these variables have been found to have a significant effect on response rates for all or some of the crocodilian populations sampled.

4.1 Breeding Period

The breeding period encapsulates the time when vulnerable eggs or hatchlings are present. This study was the first to test the effects of breeding period on crocodilian responses to distress calls.

4.1.1 Reaction time and breeding period

As predicted, the crocodilians sampled showed a significant decrease in reaction time to distress calls (- 3.32 s, 95% C.I = - 6.01, - 0.44) during the breeding period. This is in accordance with Brazaitis and Watanabe (2011), which reported an increased level of aggression in females during the breeding period. This behavioural change concurs with the hormonal differences observed in crocodilians: Kofron & Steiner (1994) reported an influx of male testosterone two months after the nesting period, a hormone which is linked to aggressive behaviour (Batrinos, 2012). Heightened levels of aggression in both sexes could account for this significant decrease in reaction time as predisposition to agnostic behaviour is greater. Increased aggression during this period makes evolutionary sense as it coincides with the offspring being most at risk from predation and thus requiring more urgent protection (Somaweera *et al.,* 2013).

4.1.2 Response rate and breeding period

Response rate to playbacks was significantly higher during the *T. schlegelii's* breeding period (20.30 higher odds, 50% C.I = 11.18, 36.86). This contradicted the previous prediction that there would be a non-significant difference in *T. schlegelii* response rate between the non-breeding and breeding period. The findings indicate that extended care is present in *T. schlegelii*, contrasting with previous reports of nest abandonment and general lack of observed care in wild populations (Britton, 2009; Vitt & Caldwell, 2009). It is likely that this contradiction arose because the *T. schlegelii* sampled in this study were captive-living and habituated to humans. Therefore, nest abandonment did not occur when the population became aware of human observers. Discussions with keepers and own observations

supported these findings. It was observed that the female's pre-playback behaviour significantly changed during the breeding period. After egg laying, the female's usual 'resting' position was on or near the nest and her responsiveness to distress calls significantly increased (Fig. 4.1). This contrasted with her 'shy' pre-breeding behaviour, largely submerged or concealed from view at the opposite end of the enclosure. The male responded to playbacks only twice and both occasions were during the breeding period. This data indicates that extended care occurs to some degree in *T. schlegelii* and is strong enough to override the effects of habituation. Further studies would be necessary, where young are kept with or near the adult's enclosure, to assess the extent of this care. This study provides the first scientific evidence of female nest guarding in *T. schlegelii*.

Contrary to expectations, both populations of *O. tetraspis* became less responsive to distress calls during the breeding period. Evidence of parental care has been reported in *O. tetraspis* (Britton, 2012). It was predicted that response rate should increase during the breeding period as it coincides with offspring being most vulnerable. Habituation to playbacks could account for this decrease. There was a significant decrease in response rates as the number of playback sessions to occur increased. This indicates a deterioration in receptibility as playback exposure increased. As the same individuals were repeatedly tested it is possible that they disassociated the playbacks with a predatory threat. The *O. tetraspis* breeding period coincided with the 6th playback session; therefore, habituation could have hindered the behavioural effects that the breeding period could induce. Playback session number was not found to influence the response rates of the other breeding populations. This indicates a species-specific difference in behaviour, as rapid habituation appeared to occur in both populations of *O. tetraspis*. An independent measures design involving a different individual for each session may have yielded a higher response rate.

Fig. 4.1. Female *T. schlegelii* resting on her nest mound during the breeding period. Taken at Crocodiles of the World, Oxford.

Interestingly, the analysis reported no significant difference in the *C. siamensis'* response rate to distress calls before and during the breeding period. If response rate to distress calls is a true measure of extended care then this result is contrary to past reports of *C. siamensis* caring for young up to a year after hatching (Conners, 2002; Bezuijen *et al.,* 2012; Sam *et al.,* 2015.). There are a few factors that could explain this discrepancy. Firstly, in this study, *C. niloticus* calls were played before and during the *C. siamensis'* breeding period. *C. siamensis* calls were only played during the breeding period and were therefore removed from this analysis. Mathevon *et al.,* (2013) reported that there should be no discrimination between different species distress calls in crocodilians. If Mathevon *et al.* (2013) conclusions were correct, then the response rate of *C. siamensis* to the two different calls should not vary. However, in this study, there were significant differences in response rate between the two species' calls, with response rate significantly increasing for own species calls (see Kin Discrimination). Species specific recognition may account for the non-significant difference in responses before and after the breeding period, as *C. siamensis* may recognise and discriminate non-kin calls. In support of this conclusion, *C. siamensis'* response rate greatly increased when distress calls of their offspring were played. Therefore, if *C. siamensis'* offspring calls were played before and during the breeding period, then a significant increase in response rate during breeding may have occurred. This is contrary to Chabert *et al.* (2015) which proposed that species specific recognition of distress calls in crocodilians is unlikely. Another possible explanation for the lack of difference in response rate during the breeding period and non-breeding period is habituation. However, the predictor variable that aims to account for

habituation (number of playback sessions) had a non-significant effect on response rate. It is still possible that rapid habituation may have occurred, but this is unlikely as the first three playback sessions stimulated no response. Finally, a non-significant difference in response rate between the non-breeding and breeding period could indicate that extended care does not occur in captive populations of *C. siamensis,* or that it varies significantly between individuals. Alternatively, response rate to distress calls may not be an accurate measure of extended care.

4.2 Sex and Care

In all populations of this study, where the sex of an individual could be identified (*C. siamensis, O. tetraspis, T. schlegelii*), there were significant differences in response rate and reaction time to distress calls. As expected, females had a higher response rate and faster reaction time in all populations studied (7.4 higher odds, 50% C.I = 3.39 – 16.18 & 6.7 s faster than males). This supports the hypothesis that response rate and reaction time are accurate measures of extended care, as the results concur with previous findings that females are the primary carer of young (Staton, 1978). The largest reported difference in response rate was between the male and female *T. schlegelii*. The male responded to a distress call on only two occasions, compared to the female's 17 times. Interestingly, response rate also significantly differed between the *O. tetraspis* populations; the distress calls provoked a higher response rate in the Bristol zoo's male than the Oxford's male. These findings support Lang's (1987) observation that crocodilians display high behavioural plasticity, which can result in significant variations between individuals of the same species.

4.3 Size of Juvenile Caller

Contrary to predictions, when the whole sample was analysed, the caller's size appeared not to significantly affect reaction time. Interestingly, the individual analysis on each population's response rate revealed that caller body size was significant but only in *O. tetraspis* and *C. rhombifer*.

As expected, the highest mean response rate for all populations sampled was elicited from the smallest juvenile (20 cm). This supports the findings of Mathevon *et al.* (2016) and Chabert *et al.* (2015), which reported higher response rates to the distress calls of smaller young. However, all three of the significant populations had a higher response rate to the 160 cm caller compared to the 60 cm caller, which disagrees with Chabert *et al.* (2015) aforementioned conclusion. A reason for this discrepancy could be that a 160 cm long crocodilian would no longer be considered juvenile and thus distress call emittance would be unusual. In relatively small species like O*. tetraspis*, a 160 cm caller would represent a mature adult. Offspring of this size would likely have dispersed naturally or been evicted from their parental

territory (Staton, 1978;Lang, 1987; Campos *et al.,* 2012). A large distress caller could represent a territorial threat or signal the presence of a bigger predator, initiating a higher response rate from the receiver. A 60 cm caller is smaller and would not be perceived as threatening and would therefore be a weaker stimulant. To support this, the two occasions where the female *T. schlegelii* (2.7 m) responded evasively was to a 160 cm long distress caller. Overall, evasive responses occurred five times and three of these occasions were in response to a 160 cm distress caller. The fourth time was in response to a 60 cm caller by a relatively small *C. niloticus* (approx. 1 m) that was near the sound source. Therefore, the relative difference in size between caller and receiver may be a greater indicator of crocodilian response rate than the caller's size alone (see Relative Size).

Contrastingly, caller body size was not significant in five of the eight populations sampled in this study. This is contrary to the Chabert *et al.* (2015) finding that fundamental pitch is a core indictor of juvenile size, which receivers use to modify their response. One possible explanation for this discrepancy is that four of the populations in this study had either not bred or were sexually immature. These crocodilians did not have young to defend; therefore, modifying response based on the distress caller's size was arguably not as important. Alternatively, there were no additional stimuli to enhance the validity that young or predators were present. Vergne *et al.* (2007) predicted that additional communicatory channels were likely being used during crocodilian distress calls. Therefore, additional olfactory or visual stimuli may have induced significant size specific differences in response (see Limitations). Another explanation for lack of significance is that size specific reactions to juvenile distress calls are not as important to crocodilian extended care as previously thought. In this study, the sex and relative size of a receiver to a caller were found to be stronger determining factors in response rate than caller body size alone.

4.4 Relative Difference in Body size Between Caller and Receiver

The relative difference in body size and its effect on response to distress calls has so far not been investigated in crocodilians. In this study, contrary to what was predicted, there was no significant difference in response strength between the two body size groups (large and small) in response to a 160 cm distress caller. Interestingly, there was a significant difference when comparing the response strength of the two groups to a 20 cm distress caller. The small-bodied group's mean response strength was double that of the large-bodied group when responding to distress callers that were 20 cm long.

A relatively small difference in body size equates to a higher chance of being predated on or threatened by the stimulus that caused the distress call emittance (Somaweera *et al.,* 2013). Individuals in the small body size group have a higher chance of succeeding in an agnostic interaction when responding to a 20 cm caller compared to a 60 cm or 160 cm caller as they are larger in size. This would explain why there

was a stronger response in the small-bodied group to the 20 cm juvenile distress calls but not to the 60 cm and 160 cm callers. Additionally, crocodilians are cannibalistic and can prey upon juveniles (Somaweera *et al.,* 2013). Preying upon a 20 cm juvenile would grant a smaller-bodied crocodilian a comparatively higher energetic gain than a larger-bodied crocodilian. A relatively higher energetic gain offers an additional motive for the smaller receivers to respond more strongly to the 20 cm juveniles compared to the larger receivers, in a cannibalistic context.

However, neither explanations account for the non-significant difference in response between the two groups to the 160 cm distress caller. Interestingly, the second strongest mean response was from the large bodied group in response to the 160 cm caller. If further tests were conducted on a larger sample size, then a significant difference in response strength between the two groups may arise. Mean pitch was the only element modified in these calls. Therefore, these results are in accordance with the Chabert *et al.* (2015) findings that when visual information is lacking, crocodilians modify their behaviour to a distress call based on the encoded size information. This is the first explorative study into the effects of relative size on crocodilian behaviour to distress calls; therefore, explanations are only speculative. Further tests are required to better understand the motives behind these responses and the effects on extended care.

4.5 Kin Discrimination

An explorative study into kin discrimination was conducted on the *C. siamensis, O. tetraspis* and *P. palpebrosus* populations. This analysis revealed a significant difference in response rate in the *C. siamensis* population between offspring and *C. niloticus* distress calls, with a higher average response rate to own species calls. This preference alludes to a species-specific recognition system, contrary to Mathevon *et al.* (2016) which reported no discrimination between different species distress calls. However, Mathevon *et al.* (2016) only sampled *Crocodylus intermedius* and *Caiman crocodilus*. Therefore, species-specific discrimination of distress calls may be used by some but not all species of crocodilians.

Chabert *et al.,* (2015) reported that information regarding species identity is encoded within crocodilian distress calls; therefore *C. siamensis* are likely using acoustic cues to optimise care towards their own young. In *C. siamensis* the temporal pattern of call utterances appears to vary distinctively from other crocodilian distress calls, with an average of five call utterances occurring in quick succession (Fig. 2.9). The distress calls of the remaining sampled species had an average two second break between each call utterance (Fig. 2.8). Environmental pressures may have caused the evolutionary divergence of *C. siamensis* distress calls, such as living sympatrically with other crocodilian species (Staniewicz *et al.,* 2018).

In accordance with Mathevon *et al.* (2016), the *O. tetraspis* population appeared to show no preference between own species and *C. niloticus* distress calls. This is likely because most crocodilian species tend not to breed at the same time and in the same micro-habitat; therefore, evolutionary pressure to distinguish between own species distress calls is low (Mathevon *et al.,* 2016).

Discrimination within own species may also occur, as it makes evolutionary sense to prioritize the distress calls of genetically related young. Both relatedness and species likely influence *C. siamensis'* responsiveness to distress calls. However, due to time constraints the influence of kin discrimination within same species calls on *C. siamensis* responses could not be conducted in this study. Therefore, the responses of the *P. palpebrosus* population to offspring and unrelated juvenile distress calls (of the same species) were investigated. Analysis of the *P. palpebrosus* population revealed a significantly higher response rate to the distress calls of offspring (45 cm) but only in comparison to the 160 cm and 60 cm unrelated juvenile calls. This contrasts with the *C. siamensis'* response rate to offspring calls, which was significantly higher than all three body lengths of the *C. niloticus* callers. These findings indicate that discrimination between distress calls may be influenced by both relatedness and caller body size.

Vergne *et al.,* (2007) predicted that, because the individual signature within crocodilian distress calls is weak, other communicatory channels are likely being utilised to identify kin. However, in this study only acoustic cues were provided. If some crocodilians can recognise own kin from distress calls alone then cues must exist within the calls that allow for this. Both the *P. palpebrosus* and *C. siamensis* populations had their eggs removed prior to hatching and had no contact with offspring thereafter. Therefore, recognition of offspring from unrelated callers of the same species could be achieved by identifying the caller signature from previous egg vocalisations, or from a species-specific call signature that parents could innately identify. Alternatively, the population may be using encoded size information within the distress calls, as juvenile body length is largely correlated to age (Chabert *et al.,* 2015). Therefore, adults may be favouring distress calls with pitches that represent juveniles of similar lengths to their offspring. To summarize, these findings are in accordance with the Lang (1987) review that species-specific behavioural differences exist in crocodilians.

4.6 Socialness of Breeding Strategy

There was a significant difference between response rate and the socialness of a species' breeding strategy in this study. However, this was only moderate ($P = 0.034$). Further experimentation that utilises a larger sample size is therefore needed. As predicted, the populations that were categorised as social breeders had a higher response strength to distress calls compared to solitary breeders. The optimal strategy for a species that forms mixed kin groups should be to respond to both kin and non-kin calls, as a predator poses a threat to all young within the mixed clutch groups. Therefore, species that engage in

social breeding strategies may be predisposed to respond to juvenile distress calls at a higher rate, due to this shared investment. Responding to non-kin calls could also have long term benefits on social species and their young, as it prevents a predator from specialising on that species and location (Trivers, 1971). Additionally, species that form social groups have a higher chance of being related to their neighbours; therefore, there is potential for genetic gain when responding to non-offspring distress calls compared to solitary species.

4.7 Species Specific Differences

Of the 24 extant species, the majority have not received adequate general scientific attention, largely due to their scarcity. This bias has created generalisations of behaviour from well-studied taxa that is not representative of the whole crocodilian phylum. To address this imbalance the sample in this study included species that have been relatively neglected by past research. Species specific differences were found in all populations within this study, regarding reaction time and response rate to distress calls. When looking at response rate, all populations differed significantly from at least three other groups. Interestingly, the *C. siamensis*, Oxford population of O. *tetraspis,* and *C. rhombifer* had significant inter-specific differences to all the other sampled groups. The two populations of *O. tetraspis* also varied significantly from each other. (P= 0.001). This finding supports the Lang (1987) review that crocodilian behaviour varies significantly, both between and within species.

4.8 Application of Research

Many species of crocodilians are under threat from habitat destruction and illegal hunting (Pough *et al.*, 2001). 47.8% of extant crocodilian species are either classified as Critically Endangered or Vulnerable (IUCN, 2019). An improved understanding of crocodilian behaviour could prevent these species being lost entirely. Additionally, crocodilians are among the last surviving members of the Archosauria clade. Combining behavioural research with molecular and morphological data could provide insight into the life histories of extinct archosaurs (Brazaitis & Watanabe, 2011).

This study aimed to sample species that are Critically Endangered or Vulnerable in order to maximise the potential benefits to conservation. By studying the Critically Endangered *T. schlegelii,* this study provides strong evidence that extended care exists in this species. Reports of parental care in the wild would indicate that un-habituated individuals do not behave naturally when humans are present, resulting in nest abandonment and a reduced ability to protect young against other predators. This study provides evidence that can inform conservation programmes of the importance of human - *T. schlegelii* segregation during breeding periods, which could be necessary for reproductive success.

Additionally, further experiments regarding the correlation between caller body size and cessation of species response could inform decisions regarding the size of young before removal from parental enclosures. This data could reduce juvenile mortality and increase the success of captive breeding programmes.

4.9 Limitations

Zoological gardens offer the unique opportunity to study Vulnerable and Endangered species, with the ability to monitor the same individuals at ease. This accessibility enables the execution of experimental methods that would be difficult to achieve in the wild. Hence, most crocodilian behavioural research has been conducted on captive populations (Lang, 1987; Brueggen, 2002; Whitaker, 2007). In captivity, resource provisioning is high and therefore cost of extended care much lower. A surplus of resources could create a higher occurrence of altruistic-like behaviours that would be sub-optimal and abnormal in the wild. However, it could be argued that as predation in captivity is virtually non-existent, incidents of protective care would be lower in comparison to the wild. To summarise, findings may not be representative of wild living crocodilians as captive environments are unnatural and lack ecological validity.

The sample size of this study was arguably small, with an average of two individuals sampled per species. Therefore, conclusions drawn are unlikely to be representative of the whole species. Additionally, crocodilian populations from the same species have been observed with significantly different behaviours, depending on the habitat they occupy (Lang, 1987). Consequently, even if the sample were much larger and consisted of wild living individuals, findings are unlikely to be generalizable to the whole species unless environmental factors and individual differences can be accounted for.

The results of this study were skewed to 0 (no response). A lack of response could indicate that care is largely absent in the sampled populations or that response rate and reaction time are not an accurate measure of extended care. Alternatively, lack of response could be due to flaws in the experimental design. Firstly, there were no visual or chemical stimuli provided in this study to signify the presence of predators or juveniles. Vergne *et al.* (2007) predicted that crocodilians rely on multiple sensory channels to communicate distress. Including additional cues, such as chemical or visual, would likely have increased the stimuli's influence on behaviour, enhancing the validity of the signal and increasing response rate (see Future Work). Due to limits on accessibility, only observable non-invasive behavioural alterations could be recorded. Therefore, physiological changes such as heart rate and cortisol levels in response to distress calls may have occurred that went undetected.

Additionally, it is unlikely that captive bred individuals have ever encountered a predator before and may therefore perceive the distress calls as false alarms. A lack of response could also be due to absent

offspring. It is possible that the Chabert *et al.* (2015) and Mathevon *et al.* (2016) experiments yielded higher response rates because their samples were comprised of breeding females that had eggs or hatchlings present. Response rate may have decreased in these studies if there were no offspring that required parental protection. Four of the eight populations in this study were non-breeding and those that did breed had their eggs removed pre-hatching.

A factor that likely accounted for a reduction in responses was habituation (Fig. 4.2). The analysis found that playback session exposure had a significant effect on response in three of the four breeding populations. The time constraints on this study meant that playback sessions could not be spaced as far apart as would be preferred. Executing playback sessions four rather than two weeks apart would have likely reduced the effects of habituation. Alternatively, an independent measures design with individuals exposed to only one playback session would prevent habituation occurring entirely. However, the successful implementation of this design would have required an immense sample size to minimise the effects of individual differences. As discussed earlier, crocodilian behaviour appears to vary significantly within individuals of the same species.

Fig. 4.2. The male and female *O. tetraspis* initially responded to the distress calls by running towards the sound source. The population responded strongly to the first playback session, but detrition of response strength occurred rapidly

4.10 Future Work

Prior to this study, research into crocodilian behaviour has been largely focused on more notable species such as *C. niloticus* and *A. mississippiensis*. This study demonstrates that behaviour can vary significantly between crocodilian species. Therefore, behavioural studies that have limited samples of just a few species are generating findings that are not representative of the whole crocodilian phylum. It

would prove valuable to replicate previous experiments like Chabert *et al.* (2015) and Mathevon *et al.* (2016) on a wider range of species. Additionally, an increased effort to assess crocodilian responses to distress calls within wild populations is important, as it would increase the validity of existing findings. However, conducting playback experiments in the field and assessing behavioural responses in relation to the predictor variables used in this study would be challenging due to wild crocodilians' elusive nature.

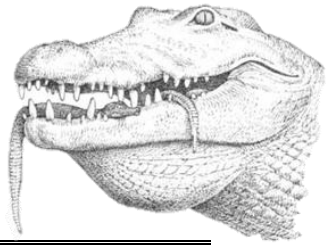
This study focuses on the immediate effects of distress calls on crocodilian behaviour and uses observational data, which is vulnerable to bias. Future work that looks at the influence of distress calls on a hormonal level would be of scientific interest, as it would show the longer-term effects that they can have on crocodilian behaviour. For example, measuring steroid hormones like cortisol, which increases in concentration in stressed individuals, would provide response data at a physiological level (Bennett & Hayssen, 2010).

Reptilian research tends to neglect alternative channels of communication, such as chemo-sensory and mechano-sensory, and is biased instead towards vision (Pough *et al.,* 2001). This paper focuses on vocal communication, but it has been discussed that crocodilians may secrete chemical signals via their mandibular glands when distressed, which conspecifics can detect (Di-Poï & Milinkovitch, 2013). Therefore, combining chemical and acoustic distress signals may stimulate stronger responses.

The lack of research into alternative functions of crocodilian distress calls other than eliciting care has been discussed (Introduction). Research on passerine birds found that distress calls can be used to startle naïve predators and increase escape rates (Conover, 1994). As their closest living relatives, crocodilian distress calls may also function to startle predators. This could be investigated by repeating the experiments of Conover (1994) and Wise *et al.* (1999) using crocodilian distress calls as opposed to those of birds.

Behavioural research into endangered and vulnerable species of crocodilian remain scarce. Any research that leads to the improvement of conservation efforts or contributes to Archosauria knowledge should be encouraged. The findings of this study suggest that behaviours related to extended care in some wild crocodilians are impacted when humans are present. This is cause for concern as crocodilian numbers continue to fall. If the trend of human environmental consumption is not curbed, then we could lose many species altogether.

5. Bibliography



Arak, A. Sexual selection by male-male competition in natterjack toad choruses. *Nature* **306**, 261–262 (1983).

Aubin, T. Why do distress calls evoke interspecific responses? An experimental study applied to some species of birds. *Behav. Process* **23**, 103–111 (1991).

Batrinos, M. L. Testosterone and aggressive behaviour in man. *Int. J. Endocr. and Meta.* **10**, 563–568 (2012).

Bennett, A. & Hayssen, V. Measuring cortisol in hair and saliva from dogs: Coat color and pigment differences. *Domest. Anim. Endocrinol.* **39**, 171–180 (2010).

Bezuijen MR, Simpson B, Behler N, D. J. & Tempsiripong Y. *Crocodylus siamensis*. IUCN Red List of Threatened Species. (2012). Available at: www.iucnredlist.org. (Accessed: 9th May 2019)

Bezuijen, M. R., Shwedick, B., Simpson, B. K., Staniewicz, A. & Stuebing, R. *Tomistoma schlegelii*. The IUCN Red List of Threatened Species. (2014). Available at: https://www.iucnredlist.org/species/21981/2780499. (Accessed: 9th May 2019)

Bezuijen, M. R., Hartoyo, P., Elliott, M. & Baker, B. A. Project *Tomistoma*: second report on the ecology of the false gharial (*Tomistoma schlegelii*) in Sumatera. **38** (1997) Wildlife Management Int. Pty. Ltd. Sanderson, Australia.

Bonke, R., Whitaker, N., Rödder, D. & Böhme, W. Vocalizations in two rare crocodilian species: A comparative analysis of distress calls of *Tomistoma schlegelii* (Müller, 1838) and Gavialis gangeticus (Gmelin, 1789). *North West. J. Zool.* **11**, 151–162 (2015).

Branch, C. L. & Freeberg, T. M. Distress calls in tufted titmice (*Baeolophus bicolor*): Are conspecifics or predators the target? *Behav. Ecol.* **23**, 854–862 (2012).

Brazaitis, P. & Watanabe, M. E. Crocodilian behaviour: A window to dinosaur behaviour? *Hist. Biol.* **23**, 73–90 (2011).

Brien, M. L., Webb, G. J., Christian, K. A., Lang, J. W. & McGuinness, K. A. Born to be bad: Agonistic behaviour in hatchling saltwater crocodiles (*Crocodylus porosus*). *Behaviour* **150**, 737–762 (2013).

Britton, A. Crocodilian Species - *Tomistoma* (*Tomistoma schlegelii*). (2009). Available at: http://crocodilian.com/cnhc/csp_tsch.htm. (Accessed: 16th May 2019)

Britton, A. *Osteolaemus tetraspis* (COPE, 1861). *Adam Britton* (2012). Available at: http://crocodilian.com/cnhc/csp_otet.htm. (Accessed: 19th August 2019)

Brown, G. E., Godin, J. G. J. & Pedersen, J. Fin-flicking behaviour: A visual antipredator alarm signal in a characin fish, *Hemigrammus erythrozonus*. *Anim. Behav.* **58**, 469–475 (1999).

Brueggen J. Crocodilians: Fact vs. Fiction. *Crocodiles Proc. 16th Work. Meet. Crocodile Spec. Gr. Species Surviv. Comm. IUCN - World Conserv. Union* 204–210 (2002).

Buffetaut, E. The Evolution of the Crocodilians. *Sci. Am.* **241**, 130–144 (1979).

Bustard, H. & Choudhury, B. Parental Care in the Salt Water Crocodile (*Crocodylus porosus*) and Management Implications. *J. Bombay Nat. Hist. Soc.* **77**, 64–69 (1980).

Campos, Z., Sanaiotti, T., Muniz, F., Farias, I. & Magnusson, W. E. Parental care in the dwarf caiman, *Paleosuchus palpebrosus* (Cuvier, 1807),*J. Nat. Hist.* **46**, 2979–2984 (2012).

Campos, Z., Sanaiotti, T. & Magnusson, W. E. Maximum size of dwarf caiman, *Paleosuchus palpebrosus* (Cuvier, 1807), in the Amazon and habitats surrounding the Pantanal, Brazil. *Amphib. Reptil.* **31**, 439–442 (2010).

Chabert, T., Colin, A., Aubin, T., Shacks, V., Bourquin, S., Elsey, R.M., Acosta, J.G. & Mathevon, N. Size does matter: Crocodile mothers react more to the voice of smaller offspring. *Sci. Rep.* **5**, 15547 (2015).

Chabrolles, L., Coureaud, G., Boyer, N., Mathevon, N. & Beauchaud, M. Cross-sensory modulation in a future top predator, the young nile crocodile. *R. Soc. Open Sci.* **4**, (2017).

Charrier, I., Jouventin, P., Mathevon, N. & Aubin, T. Individual identity coding depends on call type in the South Polar skua *Catharacta maccormicki*. *Polar Biol.* **24**, 378–382 (2001).

Chivers, D. P., Brown, G. E. & Smith, R. J. F. The evolution of chemical alarm signals: Attracting predators benefits alarm signal senders. *Am. Nat.* **148**, 649–659 (1996).

Conners, S. Parental care of Cuban crocodiles hatched at Miami Metro zoo. *Croc. Spec. Gr. Newslett.* **1**, 19–20 (2002).

Conover, M. R. Stimuli Eliciting Distress Calls in Adult Passerines and Response of Predators and Birds to Their Broadcast. *Behaviour* **131**, 19–37 (1994).

Coombs, W.P., J. Modern analogs for dinosaur nest and parenting behaviour. in *Paleobiology of the dinosaurs* (ed. Farlow, J.O) (Geological Society of America, 1989).

Crocodile Specialist Group. *Crocodylus porosus*. The IUCN Red List of Threatened Species 1996: (1996a). Available at: https://www.iucnredlist.org/species/5668/11503588. (Accessed: 9th May 2019)

Crocodile Specialist Group. *Osteolaemus tetraspis*. The IUCN Red List of Threatened Species. (1996b). Available at: https://www.iucnredlist.org/species/15635/4931429#assessment-information. (Accessed: 9th May 2019)

Crocodiles of the World. Conservation Status | Crocodiles Of The World. (2014). Available at: https://www.crocodilesoftheworld.co.uk/conservation/conservation-status/. (Accessed: 13th June 2019)

Dinets, V. Play Behavior in Crocodilians. *Anim. Behav. Cogn.* **2**, 49–55 (2015a).

Dinets, V. Apparent coordination and collaboration in cooperatively hunting crocodilians. *Ethology Ecology and Evolution* **27**, 244–250 (2015b).

Di-Poï, N. & Milinkovitch, M. C. Crocodylians evolved scattered multi-sensory micro-organs. *Evodevo* **4**, (2013).

Driver, P. M. & Humphries, D. A. the significance of the high-intensity alarm call in captured passerines. *Ibis (Lond. 1859).* **111**, 243–244 (1969).

Eckenweber, M. & Knörnschild, M. Responsiveness to conspecific distress calls is influenced by day-roost proximity in bats (*Saccopteryx bilineata*). *R. Soc. Open Sci.* **3**, 160151 (2016).

Fenton, M. B., Belwood, J. J., Fullard, J. H. & Kunz, T. H. Responses of *Myotis lucifugus* (Chiroptera: Vespertilionidae) to calls of conspecifics and to other sounds. *Can. J. Zool.* **54**, 1443–1448 (1976).

Fergusson, R. A. Nile Crocodile *Crocodylus niloticus*. *Crocodiles. Status Surv. Conserv. Action Plan* 84–89 (2010).

Fitzsimmons, N., Buchan, J., Lam, P., Polet, G., Hung, T., Thắng, N., & Gratten, J. Identification of purebred *Crocodylus siamensis* for reintroduction in Vietnam. *J. Exp. Zool.* **294**, 373–381 (John Wiley & Sons, Ltd, 2002).

Foster, K. *Tomistoma schlegelii* (On-line), Animal Diversity Web. (2013). Available at: https://animaldiversity.org/accounts/Tomistoma_schlegelii/#A9BB12F8-08B2-11E2-A35A-002500F14F28. (Accessed: 16th May 2019)

Franke, F., Schmidt, F., Borgwardt, C., Bernhard, D., Bleidorn, C., Engelmann, W., Schlegel, M. Genetic differentiation of the African dwarf crocodile *Osteolaemus tetraspis* Cope, 1861 (Crocodylia: Crocodylidae) and consequences for European zoos. *Org. Divers. Evol.* **13**, 255–266 (2013).

Fuller, R., Akite, P., Amuno, J., Fuller, C., Ofwono, J., Proaktor, G. & Ssemmanda, R. Using playback of vocalisations to survey the Nahan's francolin, a threatened African forest galliform. *Ostrich* **83**, 1–6 (2012).

Hamilton, W. D. The genetical evolution of social behaviour. *J. Theor. Biol.* **7**, 1–16 (1964).

Hassan, R., Ahmad, R., Adzhar, M., Gani, M., Ayob, A. & Zainudin, R. Notes on the Wild *Tomistoma* Populations in Western Sarawak, Malaysian Borneo. *Int. J. Ecol.* **2016**, 1–7 (2016).

Hauser, M. D. Male responsiveness to infant distress calls in free-ranging vervet monkeys. *Behav. Ecol. Sociobiol.* **19**, 65–71 (1986).Herzog, H. A. & Burghardt, G. M. Vocalization in Juvenile Crocodilians. **44**, 294–304 (1977).Hogstedt, G. Adaptation unto death: function of fear screams. *Am. Nat.* **121**, 562–570 (1983).Hutton, J. Movements, home range, dispersal and the separation of size classes in nile crocodiles. *Integr. Comp. Biol.* **29**, 1033–1049 (1989).

Isberg, S., Combrink, X., Lippai, C. & Balaguera-Reina, S. *Crocodylus niloticus*. The IUCN Red List of Threatened Species. (2019). Available at: https://www.iucnredlist.org/species/45433088/3010181. (Accessed: 9th May 2019)

IUCN 2019. The IUCN Red List of Threatened Species. Version 2019-2. http://www.iucnredlist.org. (Accessed: 10th August 2019)

Johnsen, P. B. & Wellington, J. L. Detection of Glandular Secretions by Yearling Alligators. *Copeia* **1982**, 705 (1982).

Koenig, W. D., Stanback, M. T., Hooge, P. N. & Mumme, R. L. Distress Calls in the Acorn Woodpecker. *Condor* **93**, 637–643 (1991).

Kofron, C. P. Courtship and mating of the Nile crocodile (*Crocodylus niloticus*). *Amphibia-Reptilia* **12**, 39–48 (1991).

Kofron, C. P. & Steiner, C. Observations on the African Dwarf Crocodile, *Osteolaemus tetraspis*. *Copeia* **1994**, 533 (1994).

Laiolo, P., Tella, J. L., Carrete, M., Serrano, D. & López, G. Distress calls may honestly signal bird quality to predators. *Proc. R. Soc. B Biol. Sci.* **271**, (2004).

Lang, J. W. *Social Behaviour. Crocodiles and Alligators* (Facts on File, 1989).

Lang, J. W. Crocodilian behavior: implications for management. in *Wildlife Management: Crocodiles and Alligators* (eds. Webb, G. J., Manolis, C. & Whitehead, P.) 273–294 (Surrey Beatty, 1987).

Leitch, D. B. & Catania, K. C. Structure, innervation and response properties of integumentary sensory organs in crocodilians. *J. Exp. Biol.* **215**, 4217–4230 (2012).

Lobaina, I. Evolution of maternal investment strategies for the Order Crocodylia. (2014).

Lönnstedt, O. M. & McCormick, M. I. Damsel in distress: Captured damselfish prey emit chemical cues that attract secondary predators and improve escape chances. *Proc. R. Soc. B Biol. Sci.* **282**, 20152038 (2015).

Madison, D. M. Chemical Communication in Amphibians and Reptiles. in *Chemical Signals in Vertebrates* 135–168 (Springer US, 1977). doi:10.1007/978-1-4684-2364-8_9

Magnusson, W. E., Campos, Z. & Muniz, F. *Paleosuchus palpebrosus*. The IUCN Red List of Threatened Species 2019. *The IUCN Red List of Threatened Species 2019* (2019). Available at: http://dx.doi.org/10.2305/IUCN.UK.2019-1.RLTS.T46587A3009946.en. (Accessed: 9th May 2019)

Magnusson, W. E., da Silva, E. V. & Lima, A. P. Diets of Amazonian Crocodilians. *J. Herpetol.* **21**, 85 (1987).

Magnusson, W. E., Lima, A. P., Hero, J.-M., Sanaiotti, T. M. & Yamakoshi, M. *Paleosuchus trigonatus* Nests: Sources of Heat and Embryo Sex Ratios. *J. Herpetol.* **24**, 397 (1990).

Marler, P. Characteristics of some animal calls. *Nature* **176**, (1955).

Marler, P. The logical analysis of animal communication. *J. Theor. Biol.* **1**, 295–317 (1961).

Mathevon, N., Aubin, T., Shacks, V., Bourquin, S.L., Elsey, R.M. and Acosta, J. G. The Code Size: Behavioural Response of Crocodile Mothers to Offspring Calls Depends on the Emitter's Size, Not on its Species Identity. *Crocodiles. Proc. 24th Work. Meet. IUCN-SSC Crocodile Spec. Group. IUCN Gland. Switzerland.* 79–84 (2016).

Mathevon, N., Vergne, A. & Aubin, T. Acoustic communication in crocodiles: How do juvenile calls code information? *Proceedings of Meetings on Acoustics* **19**, (2013).

Mathew, A., Ganesan, M., Majid, R. A. & Beastall, C. Breeding of False Gharial *(Tomistoma schlegelii)* at Zoo Negara, Malaysia. (2011).

Murphy, J. B., Evans, M., Augustine, L. & Miller, K. Herpetoculture. *Herpetol. Rev.* **47**, 235–240 (2016).

Nagloo, N., Collin, S. P., Hemmi, J. M. & Hart, N. S. Spatial resolving power and spectral sensitivity of the saltwater crocodile, *Crocodylus porosus,* and the freshwater crocodile, *Crocodylus johnstoni*. *J. Exp. Biol.* **219**, 1394–1404 (2016).

Passek, K. M. & Gillingham, J. C. Absence of Kin Discrimination in Hatchling American Alligators, *Alligator mississippiensis*. *Copeia* **1999**, 831 (1999).

Pfefferle, D. & Fischer, J. Sounds and size: identification of acoustic variables that reflect body size in hamadryas baboons, *Papio hamadryas*. *Anim. Behav.* **72**, 43–51 (2006).

Platt, S., Rainwater, T., Finger, A., Thorbjarnarson, J., Todd, A. & Mcmurry, S. Food habits, ontogenetic dietary partitioning and observations of foraging behaviour of Morelet's crocodile (*Crocodylus moreletii*) in northern Belize. *Herpetol. J.* **16**, 281–290 (2006).

Poel, A. M., Noach, E. J. K. & Miczek, K. A. Temporal patterning of ultrasonic distress calls in the adult rat: effects of morphine and benzodiazepines. *Psychopharmacology (Berl).* **97**, 147–148 (1989).

Pooley, A. C. & Gans, C. The Nile crocodile. *Sci. Am.* **234**, 114–119, 122 (1976).

Pough, F. H. ; Andrews, R. M ; Cadle, J. E ; Crump, M. L & Savitzky, A. H ; Wells, K.D; Ed. Pough *F.H, Herpetology.* (Prentice Hall Inc., New Jersey, 2001).

Russ, J. M., Racey, P. A. & Jones, G. Intraspecific responses to distress calls of the pipistrelle bat, *Pipistrellus pipistrellus. Anim. Behav.* **55**, 705–713 (1998).

Sam, H., Leng, H., Nhek., R., Sorn, P., Heng, S., Simpson, B., Starr, A., Brook, S. & Frechette, J. Status, distribution and ecology of the Siamese crocodile. *Cambodian Nat.Hist.* **2015**, (2015).

Semeniuk, V., Manolis, C., Webb, G. J. W. & Mawson, P. R. The Saltwater Crocodile, *Crocodylus porosus,* Schneider, 1801, in the Kimberley coastal region. *J. R. Soc. West. Aust.* **94**, 407–416 (2011).

Seyfarth, R. M., Cheney, D. L. & Marler, P. Monkey responses to three different alarm calls: Evidence of predator classification and semantic communication. *Science.* **210**, 801–803 (1980).

Shirley, M. H., Burtner, B., Oslisly, R., Sebag, D. & Testa, O. Diet and body condition of cave-dwelling dwarf crocodiles (*Osteolaemus tetraspis*, Cope 1861) in Gabon. *Afr. J. Ecol.* **55**, 411–422 (2017).

Simmons, L. W. The calling song of the field cricket, *Gryllus bimaculatus* (De Geer): constraints on transmission and its role in intermale competition and female choice. *Anim. Behav.* **36**, 380–394 (1988).

Simpson, B. K. & Bezuijen, M. R. *Crocodylus siamensis* (Siamese crocodile) **3.**Crocodile Specialist Group: Darwin. in *Crocodiles. Status Survey and Conservation Action Plan* (ed. S.C. Manolis and C. Stevenson) 120–126 (Crocodile Specialist Group, 2010).

Smith, R. J. F. Alarm signals in fishes. *Rev. Fish Biol. Fish.* **2**, 33–63 (1992).

Somaweera, R., Brien, M. & Shine, R. The Role of Predation in Shaping Crocodilian Natural History. *Herpetol. Monogr.* **27**, 23 (2013).

Staniewicz, A., Behler, N., Dharmasyah, S. & Jones, G. Niche partitioning between juvenile sympatric crocodilians in Mesangat. *Raffles Bull. Zool.* **66**, 528–537 (2018).

Staton, M. A. Distress Calls of Crocodilians --Whom Do They Benefit? *Am. Nat.* **112**, 327–332 (1978).

Stefanski, R. A. & Falls, J. B. A study of distress calls of song, swamp, and white-throated sparrows (Aves: Fringillidae). I. Intraspecific responses and functions. *Can. J. Zool.* **50**, 1501–1512 (1972).

Targarona, R. R., Soberón, R. R., Cotayo, L., Tabet, M. A. & Thorbjarnarson, J. *Crocodylus rhombifer* (errata version published in 2017). The IUCN Red List of Threatened Species (2008). Available at: http://dx.doi.org/10.2305/IUCN.UK.1996.RLTS.T5670A11516438.en. (Accessed: 9th May 2019)

Targarona, R. R., Soberón, R. R., Tabet, M. A. & Thorbjarnarson, J. B. Cuban Crocodile *Crocodylus rhombifer*. *Crocodiles. Status Surv. Conserv. Action Plan.* 114–118 (2010).

Taylor, A. M. & Reby, D. The contribution of source-filter theory to mammal vocal communication research. *J. Zool.* **280**, 221–236 (2010).

Terry, A. M., Peake, T. M. & McGregor, P. K. The role of vocal individuality in conservation. *Front. Zool.* **2**, 10 (2005).

Trivers, R. L. The evolution of reciprocal altruism. *Q. Rev. Biol.* **46**, 35–57 (1971).

Tullberg, B. S., Ah-King, M. & Temrin, H. Phylogenetic reconstruction of parental-care systems in the ancestors of birds. *Philos. Trans. R. Soc. B Biol. Sci.* **357**, 251–257 (2002).

Vergne, A. L., Pritz, M. B. & Mathevon, N. Acoustic communication in crocodilians: From behaviour to brain. *Biological Reviews* **84**, 391–411 (2009).

Vergne, A. L., Aubin, T., Taylor, P. & Mathevon, N. Acoustic signals of baby black caimans. *Zoology* **114**, 313–320 (2011).

Vergne, A. L., Avril, A., Martin, S. & Mathevon, N. Parent-offspring communication in the Nile crocodile *Crocodylus niloticus*: Do newborns' calls show an individual signature? *Naturwissenschaften* **94**, 49–54 (2007).

Vergne, A. L. & Mathevon, N. Crocodile egg sounds signal hatching time. *Current Biology* **18**, R513–R514 (2008).

Vitt, L. J. & Caldwell, J. P. *Herpetology*. (Academic Press/Elsevier, Massachusetts, USA. 2009).

Wagner, W. E. Deceptive or honest signalling of fighting ability? A test of alternative hypotheses for the function of changes in call dominant frequency by male cricket frogs. *Anim. Behav.* **44**, 449–462 (1992).

Webb, G. J. W., Messel, H. & Magnusson, W. The Nesting of *Crocodylus porosus* in Arnhem Land, Northern Australia. *Copeia* **1977**, 238 (1977).

Weldon, P. J. & Sampson, H. W. The Gular Glands of *Alligator mississippiensis*: Histology and Preliminary Analysis of Lipoidal Secretions. *Copeia* **1988**, 80 (1988).

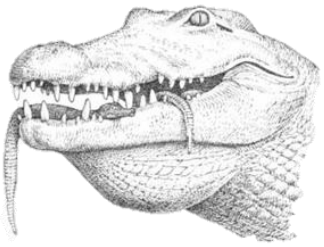
Weldon, P. J., Swenson, D. J., Olson, J. K. & Brinkmeier, W. G. The American Alligator Detects Food Chemicals in Aquatic and Terrestrial Environments. *Ethology* **85**, 191–198 (1990).

Whitaker, N. Extended parental care in the siamese crocodile (*Crocodylus siamensis*). *Russ. J. Herpetol.* **14**, 203–206 (2007).

Wise, K., Conover, M. R. & Knowlton, F. Response of coyotes to avian distress calls: Testing the startle-predator and predator-attraction hypotheses. *Behaviour* **136**, 935–949 (1999).

Zahavi, A., ed., Stonehouse, B.; Perrins. C, Reliability in communication systems and the evolution of altruism. in *Evolutionary Ecology* 253–259 (Macmillan Education, London, UK, 1977). doi:10.1007/978-1-349-05226-4_21

6. Figure Credits



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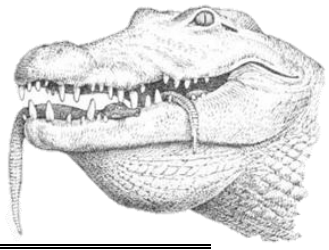
7.Appendix



Distress Calls Public Sources:

C. rhombifer: https://www.youtube.com/watch?v=yDpjYPmDvHk

C. niloticus: https://www.nature.com/articles/srep15547#supplementary-information

P. palpebrosus: https://www.youtube.com/watch?v=G3gP78P71OQ

C. porosus: "The Mutilator." *River monsters*. 10 Apr. 2011.

Human baby crying: https://www.youtube.com/watch?v=oL2B-AAnsHo